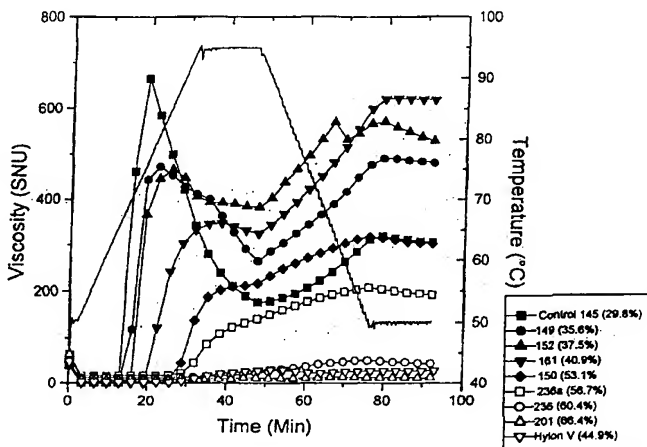




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(54) Title: IMPROVEMENTS IN OR RELATING TO PLANT STARCH COMPOSITION



## (57) Abstract

Disclosed is a nucleotide sequence encoding an effective portion of a class A starch branching enzyme (SBE) obtainable from potato plants, or a functional equivalent thereof, together with, inter alia, a corresponding polypeptide, a method of altering the characteristics of a plant, a plant having altered characteristics; and starch, particularly starch obtained from a potato plant, having novel properties.

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**Title:**        Improvements in or Relating to Plant Starch Composition

**Field of the Invention**

This invention relates to novel nucleotide sequences, polypeptides encoded thereby, vectors and host cells and host organisms comprising one or more of the novel sequences, and to a method of altering one or more characteristics of an organism. The invention also relates to starch having novel properties and to uses thereof.

**Background of the Invention**

Starch is the major form of carbon reserve in plants, constituting 50% or more of the dry weight of many storage organs - e.g. tubers, seeds of cereals. Starch is used in numerous food and industrial applications. In many cases, however, it is necessary to modify the native starches, via chemical or physical means, in order to produce distinct properties to suit particular applications. It would be highly desirable to be able to produce starches with the required properties directly in the plant, thereby removing the need for additional modification. To achieve this via genetic engineering requires knowledge of the metabolic pathway of starch biosynthesis. This includes characterisation of genes and encoded gene products which catalyse the synthesis of starch. Knowledge about the regulation of starch biosynthesis raises the possibility of "re-programming" biosynthetic pathways to create starches with novel properties that could have new commercial applications.

The commercially useful properties of starch derive from the ability of the native granular form to swell and absorb water upon suitable treatment. Usually heat is required to cause granules to swell in a process known as gelatinisation, which has been defined (W A Atwell *et al*, Cereal Foods World 33, 306-311, 1988) as "... the collapse (disruption) of molecular orders within the starch granule manifested in irreversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence, and starch solubilisation. The point of initial gelatinisation and the range over which it occurs is governed by starch concentration, method of observation, granule type, and heterogeneities within the granule population under observation". A number of techniques are available

for the determination of gelatinisation as induced by heating, a convenient and accurate method being differential scanning calorimetry, which detects the temperature range and enthalpy associated with the collapse of molecular orders within the granule. To obtain accurate and meaningful results, the peak and/or onset temperature of the endotherm observed by differential scanning calorimetry is usually determined.

The consequence of the collapse of molecular orders within starch granules is that the granules are capable of taking up water in a process known as pasting, which has been defined (W A Atwell *et al.*, Cereal Foods World 33, 306-311, 1988) as "*... the phenomenon following gelatinisation in the dissolution of starch. It involves granular swelling, exudation of molecular components from the granule, and eventually, total disruption of the granules*". The best method of evaluating pasting properties is considered to be the viscoamylograph (Atwell *et al.*, 1988 cited above) in which the viscosity of a stirred starch suspension is monitored under a defined time/temperature regime. A typical viscoamylograph profile for potato starch shows an initial rise in viscosity, which is considered to be due to granule swelling. In addition to the overall shape of the viscosity response in a viscoamylograph, a convenient quantitative measure is the temperature of initial viscosity development (onset). Figure 1 shows such a typical viscosity profile for potato starch, during and after cooking, and includes stages A-D which correspond to viscosity onset (A), maximum viscosity (B), complete dispersion (C) and reassociation of molecules (or retrogradation, D). In the figure, the dotted line represents viscosity (in stirring number units) of a 10% w/w starch suspension and the unbroken line shows the temperature in degrees centigrade. At a certain point, defined by the viscosity peak, granule swelling is so extensive that the resulting highly expanded structures are susceptible to mechanically-induced fragmentation under the stirring conditions used. With increased heating and holding at 95°C, further reduction in viscosity is observed due to increased fragmentation of swollen granules. This general profile has previously always been found for native potato starch.

After heating starches in water to 95°C and holding at that temperature (for typically 15 minutes), subsequent cooling to 50°C results in an increase in viscosity due to the process of retrogradation or set-back. Retrogradation (or set-back) is defined (Atwell *et al.*, 1988

cited above) as "...a process which occurs when the molecules comprising gelatinised starch begin to reassociate in an ordered structure...". At 50°C, it is primarily the amylose component which reassociates, as indicated by the increase in viscoamylograph viscosity for starch from normal maize (21.6% amylose) compared with starch from waxy maize (1.1% amylose) as shown in Figure 2. Figure 2 is a viscoamylograph of 10%w/w starch suspensions from waxy maize (solid line), conventional maize (dots and dashes), high amylose variety (hylon 5, dotted line) and a very high amylose variety (hylon 7, crosses). The temperature profile is also shown by a solid line, as in Figure 1. The extent of viscosity increase in the viscoamylograph on cooling and holding at 50°C depends on the amount of amylose which is able to reassociate due to its exudation from starch granules during the gelatinisation and pasting processes. A characteristic of amylose-rich starches from maize plants is that very little amylose is exuded from granules by gelatinisation and pasting up to 95°C, probably due to the restricted swelling of the granules. This is illustrated in Figure 2 which shows low viscosities for a high amylose (44.9%) starch (Hylon 5) from maize during gelatinisation and pasting at 95°C and little increase in viscosity on cooling and holding at 50°C. This effect is more extreme for a higher amylose content (58%, as in Hylon 7), which shows even lower viscosities in the viscoamylograph test (Figure 2). For commercially-available high amylose starches (currently available from maize plants, such as those described above), processing at greater than 100°C is usually necessary in order to generate the benefits of high amylose contents with respect to increased rates and strengths of reassociation, but use of such high temperatures is energetically unfavourable and costly. Accordingly, there is an unmet need for starches of high amylose content which can be processed below 100°C and still show enhanced levels of reassociation, as indicated for example by viscoamylograph measurements.

The properties of potato starch are useful in a variety of both food and non-food (paper, textiles, adhesives etc.) applications. However, for many applications, properties are not optimum and various chemical and physical modifications well known in the art are undertaken in order to improve useful properties. Two types of property manipulation which would be of use are: the controlled alteration of gelatinisation and pasting temperatures; and starches which suffer less granular fragmentation during pasting than

conventional starches.

Currently the only ways of manipulating the gelatinisation and pasting temperatures of potato starch are by the inclusion of additives such as sugars, polyhydroxy compounds of salts (Evans & Haisman, *Starke* 34, 224-231, 1982) or by extensive physical or chemical pre-treatments (e.g. Stute, *Starke* 44, 205-214, 1992). The reduction of granule fragmentation during pasting can be achieved either by extensive physical pretreatments (Stute, *Starke* 44, 205-214, 1992) or by chemical cross-linking. Such processes are inconvenient and inefficient. It is therefore desirable to obtain plants which produce starch which intrinsically possesses such advantageous properties.

Starch consists of two main polysaccharides, amylose and amylopectin. Amylose is a generally linear polymer containing  $\alpha$ -1,4 linked glucose units, while amylopectin is a highly branched polymer consisting of a  $\alpha$ -1,4 linked glucan backbone with  $\alpha$ -1,6 linked glucan branches. In most plant storage reserves amylopectin constitutes about 75% of the starch content. Amylopectin is synthesized by the concerted action of soluble starch synthase and starch branching enzyme [ $\alpha$ -1,4 glucan:  $\alpha$ -1,4 glucan 6-glycosyltransferase, EC 2.4.1.18]. Starch branching enzyme (SBE) hydrolyses  $\alpha$ -1,4 linkages and rejoins the cleaved glucan, via an  $\alpha$ -1,6 linkage, to an acceptor chain to produce a branched structure. The physical properties of starch are strongly affected by the relative abundance of amylose and amylopectin, and SBE is therefore a crucial enzyme in determining both the quantity and quality of starches produced in plant systems.

In most plants studied to date e.g. maize (Boyer & Preiss, 1978 *Biochem. Biophys. Res. Comm.* 80, 169-175), rice (Smyth, 1988 *Plant Sci.* 57, 1-8) and pea (Smith, *Planta* 175, 270-279), two forms of SBE have been identified, each encoded by a separate gene. A recent review by Burton *et al.*, (1995 *The Plant Journal* 7, 3-15) has demonstrated that the two forms of SBE constitute distinct classes of the enzyme such that, in general, enzymes of the same class from different plants may exhibit greater similarity than enzymes of different classes from the same plant. In their review, Burton *et al.* termed the two respective enzyme families class "A" and class "B", and the reader is referred thereto (and to the references cited therein) for a detailed discussion of the distinctions

between the two classes. One general distinction of note would appear to be the presence, in class A SBE molecules, of a flexible N-terminal domain, which is not found in class B molecules. The distinctions noted by Burton *et al.* are relied on herein to define class A and class B SBE molecules, which terms are to be interpreted accordingly.

However in potato, only one isoform of the SBE molecule (belonging to class B) has thus far been reported and only one gene cloned (Blennow & Johansson, 1991 *Phytochem.* 30, 437-444, and Koßmann *et al.*, 1991 *Mol. Gen. Genet.* 230, 39-44). Further, published attempts to modify the properties of starch in potato plants (by preventing expression of the single known SBE) have generally not succeeded (e.g. Müller-Rober & Koßmann 1994 *Plant Cell and Environment* 17, 601-613).

### Summary of the Invention

In a first aspect the invention provides a nucleotide sequence encoding an effective portion of a class A starch branching enzyme (SBE) obtainable from potato plants.

Preferably the nucleotide sequence encodes a polypeptide comprising an effective portion of the amino acid sequence shown in Figure 5 (excluding the sequence MNKRIDL, which does not represent part of the SBE molecule), or a functional equivalent thereof (which term is discussed below). The amino acid sequence shown in Figure 5 (Seq ID No. 15) includes a leader sequence which directs the polypeptide, when synthesised in potato cells, to the amyloplast. Those skilled in the art will recognise that the leader sequence is removed to produce a mature enzyme and that the leader sequence is therefore not essential for enzyme activity. Accordingly, an "effective portion" of the polypeptide is one which possesses sufficient SBE activity to complement the branching enzyme mutation in *E. coli* KV 832 cells (described below) and which is active when expressed in *E. coli* in the phosphorylation stimulation assay. An example of an incomplete polypeptide which nevertheless constitutes an "effective portion" is the mature enzyme lacking the leader sequence. By analogy with the pea class A SBE sequence, the potato class A sequence shown in Figure 5 probably possesses a leader sequence of about 48 amino acid residues, such that the N terminal amino acid sequence is thought to commence around the glutamic acid residue (E) at position 49 (EKSSYN... etc.). Those skilled in the art will appreciate

that an effective portion of the enzyme may well omit other parts of the sequence shown in the figure without substantial detrimental effect. For example, the C-terminal glutamic acid-rich region could be reduced in length, or possibly deleted entirely, without abolishing class A SBE activity. A comparison with other known SBE sequences, especially other class A SBE sequences (see for example, Burton *et al.* 1995 cited above), should indicate those portions which are highly conserved (and thus likely to be essential for activity) and those portions which are less well conserved (and thus are more likely to tolerate sequence changes without substantial loss of enzyme activity).

Conveniently the nucleotide sequence will comprise substantially nucleotides 289 to 2790 of the DNA sequence (Seq ID No. 14) shown in Figure 5 (which nucleotides encode the mature enzyme) or a functional equivalent thereof, and may also include further nucleotides at the 5' or 3' end. For example, for ease of expression, the sequence will desirably also comprise an in-frame ATG start codon, and may also encode a leader sequence. Thus, in one embodiment, the sequence further comprises nucleotides 145 to 288 of the sequence shown in Figure 5. Other embodiments are nucleotides 228 to 2855 of the sequence labelled "psbe2con.seq" in Figure 8, and nucleotides 57 to 2564 of the sequence shown in Figure 12 (preferably comprising an in-frame ATG start codon, such as the sequence of nucleotides 24 to 56 in the same Figure), or functional equivalents of the aforesaid sequences.

The term "functional equivalent" as applied herein to nucleotide sequences is intended to encompass those sequences which differ in their nucleotide composition to that shown in Figure 5 but which, by virtue of the degeneracy of the genetic code, encode polypeptides having identical or substantially identical amino acid sequences. It is intended that the term should also apply to sequences which are sufficiently homologous to the sequence of the invention that they can hybridise to the complement thereof under stringent hybridisation conditions - such equivalents will preferably possess at least 85%, more preferably at least 90%, and most preferably at least 95% sequence homology with the sequence of the invention as exemplified by nucleotides 289 to 2790 of the DNA sequence shown in Figure 5. It will be apparent to those skilled in the art that the nucleotide sequence of the invention may also find useful application when present as an "antisense"



sequence. Accordingly, functionally equivalent sequences will also include those sequences which can hybridise, under stringent hybridisation conditions, to the sequence of the invention (rather than the complement thereof). Such "antisense" equivalents will preferably possess at least 85%, more preferably at least 90%, and most preferably 95% sequence homology with the complement of the sequence of the invention as exemplified by nucleotides 289 to 2790 of the DNA sequence shown in Figure 5. Particular functional equivalents are shown, for example, in Figures 8 and 10 (if one disregards the various frameshift mutations noted therein).

The invention also provides vectors, particularly expression vectors, comprising the nucleotide sequence of the invention. The vector will typically comprise a promoter and one or more regulatory signals of the type well known to those skilled in the art. The invention also includes provision of cells transformed (which term encompasses transduction and transfection) with a vector comprising the nucleotide sequence of the invention.

The invention further provides a class A SBE polypeptide, obtainable from potato plants. In particular the invention provides the polypeptide in substantially pure form, especially in a form free from other plant-derived (especially potato plant-derived) components, which can be readily accomplished by expression of the relevant nucleotide sequence in a suitable non-plant host (such as any one of the yeast strains routinely used for expression purposes, e.g. *Pichia spp.* or *Saccharomyces spp.*). Typically the enzyme will substantially comprise the sequence of amino acid residues 49 to 882 shown in Figure 5 (disregarding the sequence MNKRIDL, which is not part of the enzyme), or a functional equivalent thereof. The polypeptide of the invention may be used in a method of modifying starch *in vitro*, comprising treating starch under suitable conditions (e.g. appropriate temperature, pH, etc) with an effective amount of the polypeptide according to the invention.

The term "functional equivalent", as applied herein to amino acid sequences, is intended to encompass amino acid sequences substantially similar to that shown in Figure 5, such that the polypeptide possesses sufficient activity to complement the branching enzyme mutation in *E. coli* KV 832 cells (described below) and which is active in *E. coli* in the

phosphorylation stimulation assay. Typically such functionally equivalent amino acid sequences will preferably possess at least 85%, more preferably at least 90%, and most preferably at least 95% sequence identity with the amino acid sequence of the mature enzyme (i.e. minus leader sequence) shown in Figure 5. Those skilled in the art will appreciate that conservative substitutions may be made generally throughout the molecule without substantially affecting the activity of the enzyme. Moreover, some non-conservative substitutions may be tolerated, especially in the less highly conserved regions of the molecule. Such substitutions may be made, for example, to modify slightly the activity of the enzyme. The polypeptide may, if desired, include a leader sequence, such as that exemplified by residues 1 to 48 of the amino acid sequence shown in Figure 5, although other leader sequences and signal peptides and the like are known and may be included.

A portion of the nucleotide sequence of the invention has been introduced into a plant and found to affect the characteristics of the plant. In particular, introduction of the sequence of the invention, operably linked in the antisense orientation to a suitable promoter, was found to reduce the amount of branched starch molecules in the plant. Additionally, it has recently been demonstrated in other experimental systems that "sense suppression" can also occur (i.e. expression of an introduced sequence operably linked in the sense orientation can interfere, by some unknown mechanism, with the expression of the native gene), as described by Matzke & Matzke (1995 *Plant Physiol.* 107, 679-685). Any one of the methods mentioned by Matzke & Matzke could, in theory, be used to affect the expression in a host of a homologous SBE gene.

It is believed that antisense methods are mainly operable by the production of antisense mRNA which hybridises to the sense mRNA, preventing its translation into functional polypeptide, possibly by causing the hybrid RNA to be degraded (e.g. Sheehy *et al.*, 1988 *PNAS* 85, 8805-8809; Van der Krol *et al.*, *Mol. Gen. Genet.* 220, 204-212). Sense suppression also requires homology between the introduced sequence and the target gene, but the exact mechanism is unclear. It is apparent however that, in relation to both antisense and sense suppression, neither a full length nucleotide sequence, nor a "native" sequence is essential. Preferably the "effective portion" used in the method will comprise

at least one third of the full length sequence, but by simple trial and error other fragments (smaller or larger) may be found which are functional in altering the characteristics of the plant.

Thus, in a further aspect the invention provides a method of altering the characteristics of a plant, comprising introducing into the plant an effective portion of the sequence of the invention operably linked to a suitable promoter active in the plant. Conveniently the sequence will be linked in the anti-sense orientation to the promoter. Preferably the plant is a potato plant. Conveniently, the characteristic altered relates to the starch content and/or starch composition of the plant (i.e. amount and/or type of starch present in the plant). Preferably the method of altering the characteristics of the plant will also comprise the introduction of one or more further sequences, in addition to an effective portion of the sequence of the invention. The introduced sequence of the invention and the one or more further sequences (which may be sense or antisense sequences) may be operably linked to a single promoter (which would ensure both sequences were transcribed at essentially the same time), or may be operably linked to separate promoters (which may be necessary for optimal expression). Where separate promoters are employed they may be identical to each other or different. Suitable promoters are well known to those skilled in the art and include both constitutive and inducible types. Examples include the CaMV 35S promoter (e.g. single or tandem repeat) and the patatin promoter. Advantageously the promoter will be tissue-specific. Desirably the promoter will cause expression of the operably linked sequence at substantial levels only in the tissue of the plant where starch synthesis and/or starch storage mainly occurs. Thus, for example, where the sequence is introduced into a potato plant, the operably linked promoter may be tuber-specific, such as the patatin promoter.

Desirably, for example, the method will also comprise the introduction of an effective portion of a sequence encoding a class B SBE, operably linked in the antisense orientation to a suitable promoter active in the plant. Desirably the further sequence will comprise an effective portion of the sequence encoding the potato class B SBE molecule. Conveniently the further sequence will comprise an effective portion of the sequence described by Blennow & Johansson (1991 *Phytochem.* 30, 437-444) or that disclosed in

WO92/11375. More preferably, the further sequence will comprise at least an effective portion of the sequence disclosed in International Patent Application No. WO 95/26407. Use of antisense sequences against both class A and class B SBE in combination has now been found by the present inventors to result in the production of starch having very greatly altered properties (see below). Those skilled in the art will appreciate the possibility that, if the plant already comprises a sense or antisense sequence which efficiently inhibits the class B SBE activity, introduction of a sense or antisense sequence to inhibit class A SBE activity (thereby producing a plant with inhibition of both class A and class B activity) might alter greatly the properties of the starch in the plant, without the need for introduction of one or more further sequences. Thus the sequence of the invention is conveniently introduced into plants already having low levels of class A and/or class B SBE activity, such that the inhibition resulting from the introduction of the sequence of the invention is likely to have a more pronounced effect.

The sequence of the invention, and the one or more further sequences if desired, can be introduced into the plant by any one of a number of well-known techniques (e.g. Agrobacterium-mediated transformation, or by "biolistic" methods). The sequences are likely to be most effective in inhibiting SBE activity in potato plants, but theoretically could be introduced into any plant. Desirable examples include pea, tomato, maize, wheat, rice, barley, sweet potato and cassava plants. Preferably the plant will comprise a natural gene encoding an SBE molecule which exhibits reasonable homology with the introduced nucleic acid sequence of the invention.

In another aspect, the invention provides a plant cell, or a plant or the progeny thereof, which has been altered by the method defined above. The progeny of the altered plant may be obtained, for example, by vegetative propagation, or by crossing the altered plant and reserving the seed so obtained. The invention also provides parts of the altered plant, such as storage organs. Conveniently, for example, the invention provides tubers comprising altered starch, said tubers being obtained from an altered plant or the progeny thereof. Potato tubers obtained from altered plants (or the progeny thereof) will be particularly useful materials in certain industrial applications and for the preparation and/or processing of foodstuffs and may be used, for example, to prepare low-fat waffles and

chips (amylose generally being used as a coating to prevent fat uptake), and to prepare mashed potato (especially "instant" mashed potato) having particular characteristics.

In particular relation to potato plants, the invention provides a potato plant or part thereof which, in its wild type possesses an effective SBE A gene, but which plant has been altered such that there is no effective expression of an SBE A polypeptide within the cells of at least part of the plant. The plant may have been altered by the method defined above, or may have been selected by conventional breeding to be deleted for the class A SBE gene, presence or absence of which can be readily determined by screening samples of the plants with a nucleic acid probe or antibody specific for the potato class A gene or gene product respectively.

The invention also provides starch extracted from a plant altered by the method defined above, or the progeny of such a plant, the starch having altered properties compared to starch extracted from equivalent, but unaltered, plants. The invention further provides a method of making altered starch, comprising altering a plant by the method defined above and extracting therefrom starch having altered properties compared to starch extracted from equivalent, but unaltered, plants. Use of nucleotide sequences in accordance with the invention has allowed the present inventors to produce potato starches having a wide variety of novel properties.

In particular the invention provides the following: a plant (especially a potato plant) altered by the method defined above, containing starch which, when extracted from the plant, has an elevated endotherm peak temperature as judged by DSC, compared to starch extracted from a similar, but unaltered, plant; a plant (especially a potato plant) altered by the method defined above, containing starch which, when extracted from the plant, has an elevated viscosity onset temperature (conveniently elevated by 10 - 25°C) as judged by viscoamylograph compared to starch extracted from a similar, but unaltered, plant; a plant (especially a potato plant) altered by the method defined above, containing starch which, when extracted from the plant, has a decreased peak viscosity (conveniently decreased by 240 - 700SNUs) as judged by viscoamylograph compared to starch extracted from a similar, but unaltered, plant; a plant (especially a potato plant) altered by the method

defined above, containing starch which, when extracted from the plant, has an increased pasting viscosity (conveniently increased by 37 - 260SNUs) as judged by viscoamylograph compared to starch extracted from a similar, but unaltered, plant; a plant (especially a potato plant) altered by the method defined above, containing starch which, when extracted from the plant, has an increased set-back viscosity (conveniently increased by 224 - 313 SNUs) as judged by viscoamylograph compared to starch extracted from a similar, but unaltered, plant; a plant (especially a potato plant) altered by the method defined above, containing starch which, when extracted from the plant, has a decreased set-back viscosity as judged by viscoamylograph compared to starch extracted from a similar, but unaltered, plant; and a plant (especially a potato plant) altered by the method defined above, containing starch which, when extracted from the plant, has an elevated amylose content as judged by iodometric assay (i.e. by the method of Morrison & Laignelet 1983, cited above) compared to starch extracted from a similar, but unaltered, plant. The invention also provides for starch obtainable or obtained from such plants as aforesaid.

In particular the invention provides for starch which, as extracted from a potato plant by wet milling at ambient temperature, has one or more of the following properties, as judged by viscoamylograph analysis performed according to the conditions defined below: viscosity onset temperature in the range 70-95°C (preferably 75-95°C); peak viscosity in the range 500 - 12 stirring number units; pasting viscosity in the range 214 - 434 stirring number units; set-back viscosity in the range 450 - 618 or 14 - 192 stirring number units; or displays no significant increase in viscosity during viscoamylograph. Peak, pasting and set-back viscosities are defined below. Viscosity onset temperature is the temperature at which there is a sudden, marked increase in viscosity from baseline levels during viscoamylograph, and is a term well-known to those skilled in the art.

In other particular embodiments, the invention provides starch which as extracted from a potato plant by wet milling at ambient temperature has a peak viscosity in the range 200 - 500 SNUs and a set-back viscosity in the range 275-618 SNUs as judged by viscoamylograph according to the protocol defined below; and starch which as extracted from a potato plant by wet milling at ambient temperature has a viscosity which does not decrease between the start of the heating phase (step 2) and the start of the final holding

phase (step 5) and has a set-back viscosity of 303 SNU's or less as judged by viscoamylograph according to the protocol defined below.

For the purposes of the present invention, viscoamylograph conditions are understood to pertain to analysis of a 10% (w/w) aqueous suspension of starch at atmospheric pressure, using a Newport Scientific Rapid Visco Analyser with a heating profile of: holding at 50°C for 2 minutes (step 1), heating from 50 to 95°C at a rate of 1.5°C per minute (step 2), holding at 95°C for 15 minutes (step 3), cooling from 95 to 50°C at a rate of 1.5°C per minute (step 4), and then holding at 50°C for 15 minutes (step 5). Peak viscosity may be defined for present purposes as the maximum viscosity attained during the heating phase (step 2) or the holding phase (step 3) of the viscoamylograph. Pasting viscosity may be defined as the viscosity attained by the starch suspensions at the end of the holding phase (step 3) of the viscoamylograph. Set-back viscosity may be defined as the viscosity of the starch suspension at the end of step 5 of the viscoamylograph.

In yet another aspect the invention provides starch from a potato plant having an apparent amylose content (% w/w) of at least 35%, as judged by iodometric assay according to the method described by Morrison & Laignelet (1983 *J. Cereal Science* 1, 9-20). Preferably the starch will have an amylose content of at least 40%, more preferably at least 50%, and most preferably at least 66%. Starch obtained directly from a potato plant and having such properties has not hitherto been produced. Indeed, as a result of the present invention, it is now possible to generate *in vivo* potato starch which has some properties analogous to the very high amylose starches (e.g. Hylon 7) obtainable from maize.

Starches with high (at least 35%) amylose contents find commercial application as, amongst other reasons, the amylose component of starch reassociates more strongly and rapidly than the amylopectin component during retrogradation processes. This may result, for example, in pastes with higher viscosities, gels of greater cohesion, or films of greater strength for starches with high (at least 35%) compared with normal (less than 35%) amylose contents. Alternatively, starches may be obtained with very high amylose contents, such that the granule structure is substantially preserved during heating, resulting in starch suspensions which demonstrate substantially no increase in viscosity during

cooking (i.e. there is no significant viscosity increase during viscoamylograph conditions defined above). Such starches typically exhibit a viscosity increase of less than 10% (preferably less than 5%) during viscoamylograph under the conditions defined above.

In commerce, these valuable properties are currently obtained from starches of high amylose content derived from maize plants. It would be of commercial value to have an alternative source of high amylose starches from potato as other characteristics such as granule size, organoleptic properties and textural qualities may distinguish application performances of high amylose starches from maize and potato plants.

Thus high amylose starch obtained by the method of the present invention may find application in many different technological fields, which may be broadly categorised into two groups: food products and processing; and "Industrial" applications. Under the heading of food products, the novel starches of the present invention may find application as, for example, films, barriers, coatings or gelling agents. In general, high amylose content starches absorb less fat during frying than starches with low amylose content, thus the high amylose content starches of the invention may be advantageously used in preparing low fat fried products (e.g. potato chips, crisps and the like). The novel starches may also be employed with advantage in preparing confectionery and in granular and retrograded "resistant" starches. "Resistant" starch is starch which is resistant to digestion by  $\alpha$ -amylase. As such, resistant starch is not digested by  $\alpha$ -amylases present in the human small intestine, but passes into the colon where it exhibits properties similar to soluble and insoluble dietary fibre. Resistant starch is thus of great benefit in foodstuffs due to its low calorific value and its high dietary fibre content. Resistant starch is formed by the retrogradation (akin to recrystallization) of amylose from starch gels. Such retrogradation is inhibited by amylopectin. Accordingly, the high amylose starches of the present invention are excellent starting materials for the preparation of resistant starch. Suitable methods for the preparation of resistant starch are well-known to those skilled in the art and include, for example, those described in US 5,051,271 and US 5,281,276. Conveniently the resistant starches provided by the present invention comprise at least 5% total dietary fibre, as judged by the method of Prosky *et al.*, (1985 J. Assoc. Off. Anal. Chem. 68, 677), mentioned in US 5,281, 276.



Under the heading of "Industrial" applications, the novel starches of the invention may be advantageously employed, for example, in corrugating adhesives, in biodegradable products such as loose fill packaging and foamed shapes, and in the production of glass fibers and textiles.

Those skilled in the art will appreciate that the novel starches of the invention may, if desired, be subjected *in vitro* to conventional enzymatic, physical and/or chemical modification, such as cross-linking, introduction of hydrophobic groups (e.g. octenyl succinic acid, dodecyl succinic acid), or derivatization (e.g. by means of esterification or etherification).

In yet another aspect the invention provides high (35% or more) amylose starches which generate paste viscosities greater than those obtained from high amylose starches from maize plants after processing at temperatures below 100°C. This provides the advantage of more economical starch gelatinisation and pasting treatments through the use of lower processing temperatures than are currently required for high amylose starches from maize plants.

The invention will now be further described by way of illustrative example and with reference to the drawings, of which:

Figure 1 shows a typical viscoamylograph for a 10% w/w suspension of potato starch;

Figure 2 shows viscoamylographs for 10% suspensions of starch from various maize varieties;

Figure 3 is a schematic representation of the cloning strategy used by the present inventors;

Figure 4a shows the amino acid alignment of the C-terminal portion of starch branching enzyme isoforms from various sources: amino acid residues matching the consensus

sequence are shaded;

Figure 4b shows the alignment of DNA sequences of various starch branching enzyme isoforms which encode a conserved amino acid sequence;

Figure 5 shows the DNA sequence (Seq ID No. 14) and predicted amino acid sequence (Seq ID No. 15) of a full length potato class A SBE cDNA clone obtained by PCR;

Figure 6 shows a comparison of the most highly conserved part of the amino acid sequences of potato class A (uppermost sequence) and class B (lowermost sequence) SBE molecules;

Figure 7 shows a comparison of the amino acid sequence of the full length potato class A (uppermost sequence) and pea (lowermost sequence) class A SBE molecules;

Figure 8 shows a DNA alignment of various full length potato class A SBE clones obtained by the inventors;

Figure 9 shows the DNA sequence of a potato class A SBE clone determined by direct sequencing of PCR products, together with the predicted amino acid sequence;

Figure 10 is a multiple DNA alignment of various full length potato SBE A clones obtained by the inventors;

Figure 11 is a schematic illustration of the plasmid pSJ64;

Figure 12 shows the DNA sequence and predicted amino acid sequence of the full length potato class A SBE clone as present in the plasmid pSJ90; and

Figure 13 shows viscoamylographs for 10% w/w suspensions of starch from various transgenic potato plants made by the relevant method aspect of the invention.

## Examples

### Example 1

#### **Cloning of Potato class A SBE**

The strategy for cloning the second form of starch branching enzyme from potato is shown in Figure 3. The small arrowheads represent primers used by the inventors in PCR and RACE protocols. The approximate size of the fragments isolated is indicated by the numerals on the right of the Figure. By way of explanation, a comparison of the amino acid sequences of several cloned plant starch branching enzymes (SBE) from maize (class A), pea (class A), maize (class B), rice (class B) and potato (class B), as well as human glycogen branching enzyme, allowed the inventors to identify a region in the carboxy-terminal one third of the protein which is almost completely conserved (GYLNFMGNEFGHPEWIDFPR) (Figure 4a). A multiple alignment of the DNA sequences (human, pea class A, potato class B, maize class B, maize class A and rice class B, respectively) corresponding to this region is shown in Figure 4b and was used to design an oligo which would potentially hybridize to all known plant starch branching enzymes: AATTT(C/T)ATGGGIAA(C/T)GA(A/G)TT(C/T)GG (Seq ID No. 20).

#### **Library PCR**

The initial isolation of a partial potato class A SBE cDNA clone was from an amplified potato tuber cDNA library in the  $\lambda$ Zap vector (Stratagene). One half  $\mu$ L of a potato cDNA library (titre  $2.3 \times 10^9$  pfu/mL) was used as template in a 50  $\mu$ L reaction containing 100 pmol of a 16 fold degenerate POTSBE primer and 25 pmol of a T7 primer (present in the  $\lambda$ Zap vector 3' to the cDNA sequences - see Figure 3), 100  $\mu$ M dNTPs, 2.5 U Taq polymerase and the buffer supplied with the Taq polymerase (Stratagene). All components except the enzyme were added to a 0.5 mL microcentrifuge tube, covered with mineral oil and incubated at 94°C for 7 minutes and then held at 55°C, while the Taq polymerase was added and mixed by pipetting. PCR was then performed by incubating for 1 min at 94°C, 1 min at 58°C and 3 minutes at 72°C. for 35 cycles. The PCR products were extracted with phenol/chloroform, ethanol precipitated and resuspended in TE pH 8.0 before cloning into the T/A cloning vector pT7BlueR (Invitrogen).

Several fragments between 600 and 1300 bp were amplified. These were isolated from an agarose gel and cloned into the pT7BlueR T/A cloning vector. Restriction mapping of 24 randomly selected clones showed that they belonged to several different groups (based on size and presence/absence of restriction sites). Initially four clones were chosen for sequencing. Of these four, two were found to correspond to the known potato class B SBE sequence, however the other two, although homologous, differed significantly and were more similar to the pea class A SBE sequence, suggesting that they belonged to the class A family of branching enzymes (Burton *et al.*, 1995 *The Plant Journal*, cited above). The latter two clones (~ 800bp) were sequenced fully. They both contained at the 5' end the sequence corresponding to the degenerate oligonucleotide used in the PCR and had a predicted open reading frame of 192 amino acids. The deduced amino acid sequence was highly homologous to that of the pea class A SBE.

The ~ 800 bp PCR derived cDNA fragment (corresponding to nucleotides 2281 to 3076 of the psbe2 con.seq sequence shown in Figure 8) was used as a probe to screen the potato tuber cDNA library. From one hundred and eighty thousand plaques, seven positives were obtained in the primary screen. PCR analysis showed that five of these clones were smaller than the original 800 bp cDNA clone, so these were not analysed further. The two other clones (designated 3.2.1 and 3.1.1) were approximately 1200 and 1500 bp in length respectively. These were sequenced from their 5' ends and the combined consensus sequence aligned with the sequence from the PCR generated clones. The cDNA clone 3.2.1 was excised from the phage vector and plasmid DNA was prepared and the insert fully sequenced. Several attempts to obtain longer clones from the library were unsuccessful, therefore clones containing the 5' end of the full length gene were obtained using RACE (rapid amplification of cDNA ends).

#### **Rapid Amplification of cDNA ends (RACE) and PCR conditions**

RACE was performed essentially according to Frohman (1992 *Amplifications* 11-15). Two  $\mu\text{g}$  of total RNA from mature potato tubers was heated to 65°C for 5 min and quick cooled on ice. The RNA was then reverse transcribed in a 20  $\mu\text{L}$  reaction for 1 hour at 37°C using BRL's M-MLV reverse transcriptase and buffer with 1 mM DTT, 1 mM dNTPs, 1 U/ $\mu\text{L}$  RNasin (Promega) and 500 pmol random hexamers (Pharmacia) as

primer. Excess primers were removed on a Centricon 100 column and cDNA was recovered and precipitated with isopropanol. cDNA was A-tailed in a volume of 20  $\mu$ l using 10 units terminal transferase (BRL), 200  $\mu$ M dATP for 10 min at 37°C, followed by 5 min at 65°C. The reaction was then diluted to 0.5 ml with TE pH 8 and stored at 4°C as the cDNA pool. cDNA clones were isolated by PCR amplification using the primers  $R_0R_0dT_{17}$ ,  $R_0$  and POTSBE24. The PCR was performed in 50  $\mu$ L using a hot start technique: 10  $\mu$ L of the cDNA pool was heated to 94°C in water for 5 min with 25 pmol POTSBE24, 25 pmol  $R_0$  and 2.5 pmol of  $R_0R_0dT_{17}$  and cooled to 75°C. Five  $\mu$ L of 10 x PCR buffer (Stratagene), 200  $\mu$ M dNTPs and 1.25 units of Taq polymerase were added, the mixture heated at 45°C for 2 min and 72°C for 40 min followed by 35 cycles of 94°C for 45 sec, 50°C for 25 sec, 72°C for 1.5 min and a final incubation at 72°C for 10 min. PCR products were separated by electrophoresis on 1% low melting agarose gels and the smear covering the range 600-800 bp fragments was excised and used in a second PCR amplification with 25 pmol of  $R_1$  and POTSBE25 primers in a 50  $\mu$ L reaction (28 cycles of 94°C for 1 min, 50°C 1 min, 72°C 2 min). Products were purified by chloroform extraction and cloned into pT7 Blue. PCR was used to screen the colonies and the longest clones were sequenced.

The first round of RACE only extended the length of the SBE sequence approximately 100 bases, therefore a new A-tailed cDNA library was constructed using the class A SBE specific oligo POTSBE24 (10 pmol) in an attempt to recover longer RACE products. The first and second round PCR reactions were performed using new class A SBE primers (POTSBE 28 and 29 respectively) derived from the new sequence data. Conditions were as before except that the elongation step in the first PCR was for 3 min and the second PCR consisted of 28 cycles at 94 °C for 45 seconds, 55 °C for 25 sec and 72 °C for 1 min 45 sec.

Clones ranging in size from 400 bp to 1.4 kb were isolated and sequenced. The combined sequence of the longest RACE products and cDNA clones predicted a full length gene of about 3150 nucleotides, excluding the poly(A) tail (psbe 2con.seq in Fig. 8).

As the sequence of the 5' half of the gene was compiled from the sequence of several

RACE products generated using Taq polymerase, it was possible that the compiled sequence did not represent that of a single mRNA species and/or had nucleotide sequence changes. The 5' 1600 bases of the gene was therefore re-isolated by PCR using Ulma, a thermostable DNA polymerase which, because it possesses a 3'-5' exonuclease activity, has a lower error rate compared to Taq polymerase. Several PCR products were cloned and restriction mapped and found to differ in the number of *Hind* III, *Ssp* I, and *Eco*R I sites. These differences do not represent PCR artefacts as they were observed in clones obtained from independent PCR reactions (data not shown) and indicate that there are several forms of the class A SBE gene transcribed in potato tubers.

In order to ensure that the sequence of the full length cDNA clone was derived from a single mRNA species it was therefore necessary to PCR the entire gene in one piece. cDNA was prepared according to the RACE protocol except that the adaptor oligo R<sub>6</sub>R<sub>4</sub>dT<sub>17</sub> (5 pmol) was used as a primer and after synthesis the reaction was diluted to 200  $\mu$ L with TE pH 8 and stored at 4°C. Two  $\mu$ L of the cDNA was used in a PCR reaction of 50  $\mu$ L using 25 pmol of class A SBE specific primers PBER1 and PBERT (see below), and thirty cycles of 94° for 1 min, 60°C for 1 min and 72°C for 3 min. If Taq polymerase was used the PCR products were cloned into pT7Blue whereas if Ulma polymerase was used the PCR products were purified by chloroform extraction, ethanol precipitation and kinased in a volume of 20  $\mu$ L (and then cloned into pBSSK IIP which had been cut with *Eco*RV and dephosphorylated). At least four classes of cDNA were isolated, which again differed in the presence or absence of *Hind* III, *Ssp* I and *Eco*R I sites. Three of these clones were sequenced fully, however one clone could not be isolated in sufficient quantity to sequence.

The sequence of one of the clones (number 19) is shown in Figure 5. The first methionine (initiation) codon starts a short open reading frame (ORF) of 7 amino acids which is out of frame with the next predicted ORF of 882 amino acids which has a molecular mass (Mr) of approximately 100 Kd. Nucleotides 6-2996 correspond to SBE sequence - the rest of the sequence shown is vector derived. Figure 6 shows a comparison of the most highly conserved part of the amino acid sequence of potato class A SBE (residues 180-871, top, row) and potato class B SBE (bottom row, residues 98-792); the middle row indicates the

degree of similarity, identical residues being denoted by the common letter, conservative changes by two dots and neutral changes by a single dot. Dashes indicate gaps introduced to optimise the alignment. The class A SBE protein has 44% identity over the entire length with potato class B SBE, and 56% identity therewith in the central conserved domain (Figure 6), as judged by the "Megalign" program (DNASTAR). However, Figure 7 shows a comparison between potato class A SBE (top row, residues 1-873) and pea class A SBE (bottom row, residues 1-861), from which it can be observed that cloned potato gene is more homologous to the class A pea enzyme, where the identity is 70 % over nearly the entire length, and this increases to 83 % over the central conserved region (starting at IPPP at position ~170). It is clear from this analysis that this cloned potato SBE gene belongs to the class A family of SBE genes.

An *E. coli* culture, containing the plasmid pSJ78 (which directs the expression of a full length potato SBE Class A gene), has been deposited (on 3rd January 1996) under the terms of the Budapest Treaty at The National Collections of Industrial and Marine Bacteria Limited (23 St Machar Drive, Aberdeen, AB2 1RY, United Kingdom), under accession number NCIMB 40781. Plasmid pSJ78 is equivalent to clone 19 described above. It represents a full length SBE A cDNA blunt-end ligated into the vector pBSSKIIIP.

#### Polymorphism of class A SBE genes

Sequence analysis of the other two full length class A SBE genes showed that they contain frameshift mutations and are therefore unable to encode full length proteins and indeed they were unable to complement the branching enzyme deficiency in the KV832 mutant (described below). An alignment of the full length DNA sequences is shown in Figure 8: "10con.seq" (Seq ID No. 12), "19con.seq" (Seq ID No. 14) and "11con.seq" (Seq ID No. 13) represent the sequence of full length clones 10, 19 and 11 obtained by PCR using the PBER1 and PBERT primers (see below), whilst "psbe2con.seq" (Seq ID No. 18) represents the consensus sequence of the RACE clones and cDNA clone 3.2.1. Those nucleotides which differ from the overall consensus sequence (not shown) are shaded. Dashes indicate gaps introduced to optimise the alignment. Apart from the frameshift mutations these clones are highly homologous. It should be noted that the 5' sequence of psbe2con is longer because this is the longest RACE product and it also contains several

changes compared to the other clones. The upstream methionine codon is still present in this clone but the upstream ORF is shortened to just 3 amino acids and in addition there is a 10 base deletion in the 5' untranslated leader.

The other significant area of variation is in the carboxy terminal region of the protein coding region. Closer examination of this area reveals a GAA trinucleotide repeat structure which varies in length between the four clones. These are typical characteristics of a microsatellite repeat region. The most divergent clone is #11 which has only one GAA triplet whereas clone 19 has eleven perfect repeats and the other two clones have five and seven GAA repeats. All of these deletions maintain the ORF but change the number of glutamic acid residues at the carboxy terminus of the protein.

Most of the other differences between the clones are single base changes. It is quite possible that some of these are PCR errors. To address this question direct sequencing of PCR fragments amplified from first strand cDNA was performed. Figure 9 shows the DNA sequence, and predicted amino acid sequence, obtained by such direct sequencing. Certain restriction sites are also marked. Nucleotides which could not be unambiguously assigned are indicated using standard IUPAC notation and, where this uncertainty affects the predicted amino acid sequence, a question mark is used. Sequence at the extreme 5' and 3' ends of the gene could not be determined because of the heterogeneity observed in the different cloned genes in these regions (see previous paragraph). However this can be taken as direct evidence that these differences are real and are not PCR or cloning artefacts.

There is absolutely no evidence for the frameshift mutations in the PCR derived sequence and it would appear that these mutations are an artefact of the cloning process, resulting from negative selection pressure in *E. coli*. This is supported by the fact that it proved extremely difficult to clone the full length PCR products intact as many large deletions were seen and the full length clones obtained were all cloned in one orientation (away from the LacZ promoter), perhaps suggesting that expression of the gene is toxic to the cells. Difficulties of this nature may have been responsible, at least in part, for the previous failure of other researchers to obtain the present invention.



A comparison of all the full length sequences is shown in Figure 10. In addition to clones 10, 11 and 19 are shown the sequences of a *Bgl* II - *Xho* I product cloned directly into the QE32 expression vector ("86CON.SEQ", Seq ID No. 16) and the consensus sequence of the directly sequenced PCR products ("pcrsbe2con.seq", Seq ID No. 17). Those nucleotides which differ from the consensus sequence (not shown) are shaded. Dashes indicate gaps introduced to optimise the alignment. There are 11 nucleotide differences predicted to be present in the mRNA population, which are indicated by asterisks above and below the sequence. The other differences are probably PCR artefacts or possibly sequencing errors.

#### Complementation of a branching enzyme deficient *E. coli* mutant

To determine if the isolated SBE gene encodes an active protein i.e. one that has branching enzyme activity, a complementation test was performed in the *E. coli* strain KV832. This strain is unable to make bacterial glycogen as the gene for the glycogen branching enzyme has been deleted (Keil *et al.*, 1987 Mol. Gen. Genet. 207, 294-301). When wild type cells are grown in the presence of glucose they synthesise glycogen (a highly branched glucose polymer) which stains a brown colour with iodine, whereas the KV832 cells make only a linear chain glucose polymer which stains blueish green with iodine. To determine if the cloned SBE gene could restore the ability of the KV832 cells to make a branched polymer, the clone pSJ90 (Seq ID No. 19) was used and constructed as below. The construct is a PCR-derived, substantially full length fragment (made using primers PBE 2B and PBE 2X, detailed below), which was cut with *Bgl* II and *Xho* I and cloned into the *Bam*H I / *Sal* I sites of the His-tag expression vector pQE32 (Qiagen). This clone, pSJ86, was sequenced and found to have a frameshift mutation of two bases in the 5' half of the gene. This frameshift was removed by digestion with *Nsi* I and *Sna*B I and replaced with the corresponding fragment from a Taq-generated PCR clone to produce the plasmid pSJ90 (sequence shown in Figure 12; the first 10 amino acids are derived from the expression vector). The polypeptide encoded by pSJ90 would be predicted to correspond to amino acids 46-882 of the full SBE coding sequence. The construct pSJ90 was transformed into the branching enzyme deficient KV832 cells and transformants were grown on solid PYG medium (0.85%  $\text{KH}_2\text{PO}_4$ , 1.1%  $\text{K}_2\text{HPO}_4$ , 0.6% yeast extract) containing 1.0% glucose. To test for complementation, a loop of cells was

scraped off and resuspended in 150 $\mu$ l of water. to which was added 15 $\mu$ l Lugol's solution (2g KI and 1g I<sub>2</sub> per 300ml water). It was found that the potato SBE fragment-transformed KV832 cells now stained a yellow-brown colour with iodine whereas control cells containing only the pQE32 vector continued to stain blue-green.

#### Expression of potato class A SBE in *E. coli*

Single colonies of KV832, containing one of the plasmids pQE32, pAGCR1 or pSJ90, were picked into 50ml of 2xYT medium containing carbenicillin, kanamycin and streptomycin as appropriate (100, 50 and 25 mg/L, respectively) in a 250ml flask and grown for 5 hours, with shaking, at 37°C. IPTG was then added to a final concentration of 1mM to induce expression and the flasks were further incubated overnight at 25°C. The cells were harvested by centrifugation and resuspended in 50 mM sodium phosphate buffer (pH 8.0), containing 300mM NaCl, 1mg/ml lysozyme and 1mM PMSF and left on ice for 1 hour. The cell lysates were then sonicated (3 pulses of 10 seconds at 40% power using a microprobe) and cleared by centrifugation at 12,000g for 10 minutes at 4°C. Cleared lysates were concentrated approximately 10 fold in a Centricon™ 30 filtration unit. Duplicate 10 $\mu$ l samples of the resulting extract were assayed for SBE activity by the phosphorylation stimulation method, as described in International Patent Application No. PCT/GB95/00634. In brief, the standard assay reaction mixture (0.2ml) was 200mM 2-(N-morpholino) ethanesulphonic acid (MES) buffer pH6.5, containing 100nCi of <sup>14</sup>C glucose-1-phosphate at 50mM, 0.05 mg rabbit phosphorylase A, and *E. coli* lysate. The reaction mixture was incubated for 60 minutes at 30°C and the reaction terminated and glucan polymer precipitated by the addition of 1ml of 75% (v/v) methanol, 1% (w/v) potassium hydroxide, and then 0.1ml glycogen (10mg/ml). The results are presented below:

Construct	SBE Activity (cpm)
pQE32 (control)	1,829
pSJ90 (potato class A SBE)	14,327
pAGCR1 (pea class A SBE)	29,707

The potato class A SBE activity is 7-8 fold above background levels. It was concluded therefore that the potato class A SBE gene was able to complement the BE mutation in the

phosphorylation stimulation assay and that the cloned gene does indeed code for a protein with branching enzyme activity.

### Oligonucleotides

The following synthetic oligonucleotides (Seq ID No.s 1-11 respectively) were used:

R <sub>0</sub> R <sub>1</sub> dT <sub>17</sub>	AAGGATCCGTCGACATCGATAATACGACTCACTATAGGGA(T) <sub>17</sub>
R <sub>0</sub>	AAGGATCCGTCGACATC
R <sub>1</sub>	GACATCGATAATACGAC
POTSBE24	CATCCAACCACCATCTCGCA
POTSBE25	TTGAGAGAAGATACCTAAGT
POTSBE28	ATGTTCAGTCCATCTAAAGT
POTSBE29	AGAACAACAATTCCTAGCTC
PBER 1	GGGGCCTTGAAGTCAGCAAT
PBERT	CGTCCCAGCATTTCGACATAA
PBE 2B	CTTGGATCCTTGAAGTCAGCAATTTG
PBE 2X	TAACTCGAGCAACGCGATCACAAGTTCGT

### Example 2

#### Production of Transgenic Plants

##### Construction of plant transformation vectors with antisense starch branching enzyme genes

A 1200 bp *Sac* I - *Xho* I fragment, encoding approximately the -COOH half of the potato class A SBE (isolated from the rescued  $\lambda$ Zap clone 3.2.1), was cloned into the *Sac* I - *Sal* I sites of the plant transformation vector pSJ29 to create plasmid pSJ64, which is illustrated schematically in Figure 11. In the figure, the black line represents the DNA sequence. The broken line represents the bacterial plasmid backbone (containing the origin of replication and bacterial selection marker), which is not shown in full. The filled triangles on the line denote the T-DNA borders (RB = right border, LB = left border). Relevant restriction sites are shown above the black line, with the approximate distances (in kilobases) between the sites (marked by an asterisk) given by the numerals below the

line. The thinnest arrows indicate polyadenylation signals (pAnos = nopaline synthase, pAg7 = Agrobacterium gene 7), the arrows intermediate in thickness denote protein coding regions (SBE II = potato class A SBE, HYG = hygromycin resistance gene) and the thickest arrows represent promoter regions (P-2x35 = double CaMV 35S promoter, Pnos = nopaline synthase promoter). Thus pSJ64 contained the class A SBE gene fragment in an antisense orientation between the 2X 35S CaMV promoter and the nopaline synthase polyadenylation signal.

For information, pSJ29 is a derivative of the binary vector pGPTV-HYG (Becker *et al.*, 1992 Plant Molecular Biology 20, 1195-1197) modified as follows: an approximately 750 bp (*Sac* I, T4 DNA polymerase blunted - *Sal* I) fragment of pJIT60 (Guerineau *et al.*, 1992 Plant Mol. Biol. 18, 815-818) containing the duplicated cauliflower mosaic virus (CaMV) 35S promoter (Cabb-JI strain, equivalent to nucleotides 7040 to 7376 duplicated upstream of 7040 to 7433, Frank *et al.*, 1980 Cell 21, 285-294) was cloned into the *Hind* III (Klenow polymerase repaired) - *Sal* I sites of pGPTV-HYG to create pSJ29.

### Plant transformation

Transformation was conducted on two types of potato plant explants; either wild type untransformed minitubers (in order to give single transformants containing the class A antisense construct alone) or minitubers from three tissue culture lines (which gave rise to plants #12, #15, #17 and #18 indicated in Table 1) which had already been successfully transformed with the class B (SBE I) antisense construct containing the tandem 35S promoter (so as to obtain double transformant plants, containing antisense sequences for both the class A and class B enzymes).

Details of the method of Agrobacterium transformation, and of the growth of transformed plants, are described in International Patent Application No. WO 95/26407, except that the medium used contained 3% sucrose (not 1%) until the final transfer and that the initial incubation with Agrobacterium (strain 3850) was performed in darkness. Transformants containing the class A antisense sequence were selected by growth in medium containing 15mg/L hygromycin (the class A antisense construct comprising the HYG gene, i.e. hygromycin phosphotransferase).

Transformation was confirmed in all cases by production of a DNA fragment from the antisense gene after PCR in the presence of appropriate primers and a crude extract of genomic DNA from each regenerated shoot.

### Characterisation of starch from potato plants

Starch was extracted from plants as follows: potato tubers were homogenised in water for 2 minutes in a Waring blender operating at high speed. The homogenate was washed and filtered (initially through 2mm, then through 1mm filters) using about 4 litres of water per 100gms of tubers (6 extractions). Washed starch granules were finally extracted with acetone and air dried.

Starch extracted from singly transformed potato plants (class A/SBE II antisense, or class B/SBE I antisense), or from double transformants (class A/SBE II and class B/SBE I antisense), or from untransformed control plants, was partially characterised. The results are shown in Table 1. The table shows the amount of SBE activity (units/gram tissue) in tubers from each transformed plant. The endotherm peak temperature (°C) of starch extracted from several plants was determined by DSC, and the onset temperature (°C) of pasting was determined by reference to a viscoamylograph ("RVA"), as described in WO 95/26407. The viscoamylograph profile was as follows: step 1 - 50°C for 2 minutes; step 2 - increase in temperature from 50°C to 95°C at a rate of 1.5°C per minute; step 3 - holding at 95°C for 15 minutes; step 4 - cooling from 95°C to 50°C at a rate of 1.5°C per minute; and finally, step 5 - holding at 50°C for 15 minutes. Table 1 shows the peak, pasting and set-back viscosities in stirring number units (SNUs), which is a measure of the amount of torque required to stir the suspensions. Peak viscosity may be defined for present purposes as the maximum viscosity attained during the heating phase (step 2) or the holding phase (step 3) of the viscoamylograph. Pasting viscosity may be defined as the viscosity attained by the starch suspensions at the end of the holding phase (step 3) of the viscoamylograph. Set-back viscosity may be defined as the viscosity of the starch suspension at the end of step 5 of the viscoamylograph.

A determination of apparent amylose content (% w/w) was also performed, using the iodometric assay method of Morrison & Laignelet (1983 *J. Cereal Sci.* 1, 9-20). The

results (percentage apparent amylose) are shown in Table 1. The untransformed and transformed control plants gave rise to starches having apparent amylose contents in the range 29(+/-3)%.

Generally similar values for amylose content were obtained for starch extracted from most of the singly transformed plants containing the class A (SBE II) antisense sequence. However, some plants (#152, 249) gave rise to starch having an apparent amylose content of 37-38%, notably higher than the control value. Starch extracted from these plants had markedly elevated pasting onset temperatures, and starch from plant 152 also exhibited an elevated endotherm peak temperature (starch from plant 249 was not tested by DSC).

Table 1

Sample description	Sample number	Tuber SBE activity (Mg starch)	DSC		Viscometry graph			RVN		Apparent amylose content (% w/w)	Phosphorus content (mg/100g)
			Peak temperature (°C)		Onset temperature (°C)	Peak viscosity (SBU)	Pasting viscosity (SBU)	Set-back viscosity (SBU)			
Untransformed control	148	7.8	85.8		85.5	645	181	280	31.2	68	
	243	22.2	nd		82.6	781	135	241	29.1		
AS-Class A SBE	152	12.7	88.5		70.8	467	300	528	37.5	68	
	249	13.8	nd		70.0	487	434	518	38.5		
AS-Class B SBE (17) (control)	145	0.7	88.9		88.8	699	177	305	29.8	111	
AS-Class B SBE (17) + AS-Class A SBE	150	0.6	74.0		80.0	214	214	303	53.1	188	
	181	0.5	73.0		78.8	348	324	618	40.9	208	
AS-Class B SBE (18) (control)	144	1.8	84.5		84.7	714	154	258	28.0	97	
AS-Class B SBE (18) + AS-Class A SBE	149	3.0	88.5		88.9	474	287	482	35.8	127	
AS-Class B SBE (15) (control)	172	0.22	nd		85.4	707	187	280	28.8	130	
AS-Class B SBE (15) + AS-Class A SBE	201	0.10	nd		>95	no peak	12	13	88.4	210	
	208a	0.10	nd		>95	no peak	15	17	84.1		
	208	0.30	72.8-80.5		>95	no peak	14	19	82.8	240	
	202	0.02	nd		88.4	no peak	172	245	57.8		
	212	1.40	nd		78.0	308	268	541	48.5		
220	1.40	nd		75.8	355	345	583	44.1			
AS-Class B SBE (12) (control)	170	0.2	nd		88.5	768	202	303	27.8		
AS-Class B SBE (12) + AS-Class A SBE	238	0.7	nd		85.0	no peak	23	14	80.4		
	238a	0.9	nd		91.2	no peak	138	182	56.7		
	230a	0.8	nd		77.8	244	238	450	48.2		

50°C (2 min), 50-95°C (1.5°C/min), 95°C (15 min), 95-50°C (1.5°C/min), 50°C (18 min)

at end of 50°C (2min), 50-95°C (1.5°C/min), 95°C (15 min)

at end of profile

RVN profile

Pasting viscosity (47 min)

Setback viscosity (82 min)

SBE

SBU

nd

Starch Branching Enzyme

Instrument "Siring Number Units" (arbitrary units)

not determined

29/1

Table 1

Sample description	Sample number	Tuber SBE activity (U/g starch)	DSC	
			Peak temperature (°C)	Onset temperature (°C)
Untransformed control	146	7.6	65.8	65.5
	243	22.2	nd	62.6
AS-Class A SBE	152	12.7	69.5	70.9
	249	13.9	nd	70.0
AS-Class B SBE (17) (control)	145	0.7	66.9	66.8
AS-Class B SBE (17) + AS-Class A SBE	150	0.6	74.0	86.0
	161	0.5	73.0	76.6
AS-Class B SBE (18) (control)	144	1.6	64.5	64.7
AS-Class B SBE (18) + AS-Class A SBE	149	3.0	68.5	69.9



29/2

Viscosimetryograph			Apparent amylose content (% w/w)	Phosphorus content (mg/100g)
(RVA)				
Peak viscosity (SNU)	Pasting viscosity (SNU)	Set-back viscosity (SNU)		
545	181	280	31.2	68
761	135	241	29.1	
467	380	520	37.5	89
497	434	518	38.5	
669	177	305	29.8	111
214	214	303	53.1	198
349	324	618	40.9	206
714	154	258	29.0	97
474	267	482	35.6	127

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AS-Class B SBE (15) (control)	172	0.22	nd	65.4
AS-Class B SBE (15) + AS-Class A SBE	201	0.10	nd	>95
	208a	0.10	nd	>95
	208	0.30	72.8-80.5	>95
	202	0.02	nd	89.4
	212	1.40	nd	78.0
	220	1.40	nd	75.8
AS-Class B SBE (12) (control)	170	0.2	nd	66.5
AS-Class B SBE (12) + AS-Class A SBE	236	0.7	nd	95.0
	236a	0.9	nd	91.2
	230a	0.8	nd	77.6

RVA profile

Pasting viscosity (47 min)

Set-back viscosity (92 min)

SBE

SNU

nd

50°C (2 min), 50-95°C (1.5°C/min), 95°C (15 min), 95-50°C (1.5°C/min), 50°C (15 min)

at end of 50°C (2min), 50-95°C (1.5°C/min), 95°C (15 min)

at end of profile

Starch Branching Enzyme

Instrument "Stirring Number Units" (arbitrary units)

not determined

29/4

707	167	290	28.8	130
no peak	12	13	66.4	210
no peak	15	17	64.1	
no peak	14	19	62.8	240
no peak	172	245	57.9	
308	296	541	49.5	
355	345	593	44.1	
768	202	303	27.8	
no peak	23	14	60.4	
no peak	139	192	56.7	
244	239	450	48.2	

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It should be noted that, even if other single transformants were not to provide starch with an altered amylose/amylopectin ratio, the starch from such plants might still have different properties relative to starch from conventional plants (e.g. different average molecular weight or different amylopectin branching patterns), which might be useful.

Double transformant plants, containing antisense sequences for both the class A and class B enzymes, had greatly reduced SBE activity (units/gm) compared to untransformed plants or single anti-sense class A transformants, (as shown in Table 1). Moreover, certain of the double transformant plants contained starch having very significantly altered properties. For example, starch extracted from plants #201, 202, 208, 208a, 236 and 236a had drastically altered amylose/amylopectin ratios, to the extent that amylose was the main constituent of starch from these plants. The pasting onset temperatures of starch from these plants were also the most greatly increased (by about 25-30°C). Starch from plants such as #150, 161, 212, 220 and 230a represented a range of intermediates, in that such starch displayed a more modest rise in both amylose content and pasting onset temperature. The results would tend to suggest that there is generally a correlation between % amylose content and pasting onset temperature, which is in agreement with the known behaviour of starches from other sources, notably maize.

The marked increase in amylose content obtained by inhibition of class A SBE alone, compared to inhibition of class B SBE alone (see PCT/GB95/00634) might suggest that it would be advantageous to transform plants first with a construct to suppress class A SBE expression (probably, in practice, an antisense construct), select those plants giving rise to starch with the most altered properties, and then to re-transform with a construct to suppress class B SBE expression (again, in practice, probably an antisense construct), so as to maximise the degree of starch modification.

In addition to pasting onset temperatures, other features of the viscoamylograph profile e.g. for starches from plants #149, 150, 152, 161, 201, 236 and 236a showed significant differences to starches from control plants, as illustrated in Figure 13. Referring to Figure 13, a number of viscoamylograph traces are shown. The legend is as follows: shaded box - normal potato starch control (29.8% amylose content): shaded circle - starch from plant

149 (35.6% amylose): shaded triangle, pointing upwards - plant 152 (37.5%); shaded triangle, pointing downwards - plant 161 (40.9%); shaded diamond - plant 150 (53.1%); unshaded box - plant 236a (56.7%); unshaded circle - plant 236 (60.4%); unshaded triangle, pointing upwards - plant 201 (66.4%); unshaded triangle, pointing downwards - Hylon V starch, from maize (44.9 % amylose). The thin line denotes the heating profile.

With increasing amylose content, peak viscosities during processing to 95°C decrease, and the drop in viscosity from the peak until the end of the holding period at 95°C also generally decreases (indeed, for some of the starch samples there is an increase in viscosity during this period). Both of these results are indicative of reduced granule fragmentation, and hence *increased* granule stability during pasting. This property has not previously been available in potato starch without extensive prior chemical or physical modification. For applications where a maximal viscosity after processing to 95°C is desirable (i.e. corresponding to the viscosity after 47 minutes in the viscoamylograph test), starch from plant #152 would be selected as starches with both lower (Controls, #149) and higher (#161, #150) amylose contents have lower viscosities following this gelatinisation and pasting regime (Figure 13 and Table 1). It is believed that the viscosity at this stage is determined by a combination of the extent of granule swelling and the resistance of swollen granules to mechanical fragmentation. For any desired viscosity behaviour, one skilled in the art would select a potato starch from a range containing different amylose contents produced according to the invention by performing suitable standard viscosity tests.

Upon cooling pastes from 95°C to 50°C, potato starches from most plants transformed in accordance with the invention showed an increase in viscoamylograph viscosity as expected for partial reassociation of amylose. Starches from plants #149, 152 and 161 all show viscosities at 50°C significantly in excess of those for starches from control plants (Figure 13 and Table 1). This contrasts with the effect of elevated amylose contents in starches from maize plants (Figure 2) which show very low viscosities throughout the viscoamylograph test. Of particular note is the fact that, for similar amylose contents, starch from potato plant 150 (53% amylose) shows markedly increased viscosity compared with Hylon 5 starch (44.9% amylose) as illustrated in Figure 13. This demonstrates that

useful properties which require elevated (35% or greater) amylose levels can be obtained by processing starches from potato plants below 100°C, whereas more energy-intensive processing is required in order to generate similarly useful properties from high amylose starches derived from maize plants.

Final viscosity in the viscoamylograph test (set-back viscosity after 92 minutes) is greatest for starch from plant #161 (40.9% amylose) amongst those tested (Figure 13 and Table 1). Decreasing final viscosities are obtained for starches from plant #152 (37.5% amylose), #149 (35.6% amylose) and #150 (53.1% amylose). Set-back viscosity occurs where amylose molecules, exuded from the starch granule during pasting, start to re-associate outside the granule and form a viscous gel-like substance. It is believed that the set-back viscosity values of starches from transgenic potato plants represent a balance between the inherent amylose content of the starches and the ability of the amylose fraction to be exuded from the granule during pasting and therefore be available for the reassociation process which results in viscosity increase. For starches with low amylose content, increasing the amylose content tends to make more amylose available for re-association, thus increasing the set-back viscosity. However, above a threshold value, increased amylose content is thought to inhibit granule swelling, thus preventing exudation of amylose from the starch granule and reducing the amount of amylose available for re-association. This is supported by the RVA results obtained for the very high amylose content potato starches seen in the viscoamylograph profiles in Figure 13. For any desired viscosity behaviour following set-back or retrogradation to any desired temperature over any desired timescale, one skilled in the art would select a potato starch from a range containing different amylose contents produced according to the invention by performing standard viscosity tests.

Further experiments with starch from plants #201 and 208 showed that this had an apparent amylose content of over 62% (see Table 1). Viscoamylograph studies showed that starch from these plants had radically altered properties and behaved in a manner similar to hylon 5 starch from maize plants (Figure 13). Under the conditions employed in the viscoamylograph, this starch exhibited extremely limited (nearly undetectable) granule swelling. Thus, for example, unlike starch from control plants, starch from plants

201, 208 and 208a did not display a clearly defined pasting viscosity peak during the heating phase. Microscopic analysis confirmed that the starch granule structure underwent only minor swelling during the experimental heating process. This property may well be particularly useful in certain applications, as will be apparent to those skilled in the art.

Some re-grown plants have so far been found to increase still further the apparent amylose content of starch extracted therefrom. Such increases may be due to:-

- i) Growth and development of the first generation transformed plants may have been affected to some degree by the exogenous growth hormones present in the tissue culture system, which exogenous hormones were not present during growth of the second generation plants; and
- ii) Subsequent generations were grown under field conditions, which may allow for attainment of greater maturity than growth under laboratory conditions, it being generally held that amylose content of potato starch increases with maturity of the potato tuber. Accordingly, it should be possible to obtain potato plants giving rise to tubers with starch having an amylose content in excess of the 66% level so far attained, simply by analysing a greater number of transformed plants and/or by re-growing transgenic plants through one or more generations under field conditions.

Table 1 shows that another characteristic of starch which is affected by the presence of anti-sense sequences to SBE is the phosphorus content. Starch from untransformed control plants had a phosphorus content of about 60-70mg/100gram dry weight (as determined according to the AOAC Official Methods of Analysis, 15th Edition, Method 948.09 "Phosphorus in Flour"). Introduction into the plant of an anti-sense SBE B sequence was found to cause a modest increase (about two-fold) in phosphorus content, which is in agreement with the previous findings reported at scientific meetings. Similarly, anti-sense to SBE A alone causes only a small rise in phosphorus content relative to untransformed controls. However, use of anti-sense to both SBE A and B in combination results in up to a four-fold increase in phosphorus content, which is far greater than any *in planta* phosphorus content previously demonstrated for potato starch.

This is useful in that, for certain applications, starch must be phosphorylated *in vitro* by

chemical modification. The ability to obtain potato starch which, as extracted from the plant, already has a high phosphorus content will reduce the amount of *in vitro* phosphorylation required suitably to modify the starch. Thus, in another aspect the invention provides potato starch which, as extracted from the plant, has a phosphorus content in excess of 200mg/100gram dry weight starch. Typically the starch will have a phosphorus content in the range 200 - 240mg/100gram dry weight starch.



## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT:

- (A) NAME: National Starch and Chemical Investment  
Holding Corporation
- (B) STREET: 501 Silverside Road, Suite 27
- (C) CITY: Wilmington
- (D) STATE: Delaware
- (E) COUNTRY: United States of America
- (F) POSTAL CODE (ZIP): 19809

(ii) TITLE OF INVENTION: Improvements in or Relating to Plant Starch  
Composition

(iii) NUMBER OF SEQUENCES: 20

## (iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

AAGGATCCGT CGACATCGAT AATACGACTC ACTATAGGGA TTTTTTTTTT TTTTTT

57

## (2) INFORMATION FOR SEQ ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

AAGGATCCGT CGACATC

17

## (2) INFORMATION FOR SEQ ID NO: 3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs

(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GACATCGATA ATACGAC

17

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CATCCAACCA CCATCTCGCA

20

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

TTGAGAGAAG ATACCTAAGT

20

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ATGTTGAGTC CATCTAAAGT

20

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

AGAACAACAA TTCCTAGCTC

20

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GGGGCCTTGA ACTCAGCAAT

20

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CGTCCCAGCA TTCGACATAA

20

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CTTGGATCCT TGAATCAGC AATTTG

26

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

TAACTCGAGC AACGCGATCA CAAGTTCGT

29

## (2) INFORMATION FOR SEQ ID NO: 12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3003 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GATGGGGCCT TGAAC	TGACAG	AATTTGACAC	TCAGTTAGTT	ACACTGCCAT	CACTTATCAG	60
ATCTCTATTT TTTCT	CTTAA	TTCCAACCAA	GGAATGAATA	AAAAGATAGA	TTTGTA	120
CCCTAAGGAG	AGAAGAAGAA	AGATGGTGTA	TACACTCTCT	GGAGTTCGTT	TTCTACTGT	180
TCCATCAGTG	TACAAATCTA	ATGGATTCAG	CAGTAATGGT	GATCGGAGGA	ATGCTAATAT	240
TTCTGTATTC	TTGAAAAAC	ACTCTCTTTC	ACGGAAGATC	TTGGCTGAAA	AGTCTTCTTA	300
CAATTCCGAA	TCCCACCTT	CTACAATTGC	AGCATCGGGG	AAAGTCCTTG	TGCCTGGAAT	360
CCAGAGTGAT	AGCTCCTCAT	CCTCAACAGA	TCAATTTGAG	TTGCTGAGA	CATCTCCAGA	420
AAATTCCTCA	GCATCAACTG	ATGTAGATAG	TTCAACAATG	GAACACGCTA	GCCAGATTAA	480
AACTGAGAAC	GATGACGTTG	AGCCGTCAAG	TGATCTTACA	GGAAGTGTG	AAGAGCTGGA	540
TTTTGCTTCA	TACTACAAC	TACAAGAAGG	TGGTAACTG	GAGGAGTCTA	AAACATTAAA	600
TACTTCTGAA	GAGACAATTA	TTGATGAATC	TGATAGGATC	AGAGAGAGGG	GCATCCCTCC	660
ACCTGGACTT	GGTCAGAAGA	TTTATGAAAT	AGACCCCTT	TTGACAACT	ATCGTCAACA	720
CCTTGATTAC	AGGTATTCAC	AGTACAAGAA	ACTGAGGGAG	GCAATTGACA	AGTATGAGGG	780
TGGTTTGGAA	GCTTTTTTCT	GTGGTTATGA	AAGAATGGGT	TTCACTCGTA	GTGCTACAGG	840
TATCACTTAC	CGTGAGTGGG	CTCCTGGTGC	CCAGTCAGCT	GCCCTCATTG	GGGATTTCAA	900
CAATTGGGAC	GCAATGCTG	ACTTTATGAC	TCGGAATGAA	TTTGGTGTCT	GAGAGATTTT	960
TCTGCCAAAT	AATGTGGATG	GTTCTCCTGC	AATTCCTCAT	GGGTCCAGAG	TGAAGATACG	1020
TATGGACACT	CCATCAGGTG	TTAAGGATTC	CATTCTGCT	TGGATCAACT	ACTCTTTACA	1080
GCTTCTGAT	GAAATCCAT	ATAATGGAAT	ATATTATGAT	CCACCCGAAG	AGGAGAGGTA	1140
TATCTTCCAA	CACCCACGGC	CAAAGAAACC	AAAGTCGGTG	AGAATATATG	AATCTCATAT	1200
TGGAATGAGT	AGTCCGGAGC	CTAAAATTAA	CTCATACGTG	AATTTTAGAG	ATGAAGTTCT	1260
TCCTCGCATA	AAAAAGCTT	GGGTACAATG	CGGTGCAAAT	TATGGCTATT	CAAGAGCATT	1320
CTTATTATGC	TAGTTTTTGGT	TATCATGTCA	CAAATTTTTT	TGCACCAAGC	AGCCGTTTTG	1380

GAACGCCGA CGACCTTAAG TCTTTGATTG ATAAAGCTCA TGAGCTAGGA ATTGTTGTTT	1440
TCATGGACAT TGTTACACAGC CATGCATCAA ATAATACTTT AGATGGACTG AACATGTTTG	1500
ACGGCACAGA TAGTTGTTAC TTTCACCTCG GAGCTCGTGG TTATCATTTG ATGTGGGATT	1560
TCCGCCTCTT TAACTATGGA AACTGGGAGG TACTTAGGTA TCTTCTCTCA AATGCGAGAT	1620
GGTGGTTGGA TGAGTTCAAA TTTGATGGAT TTAGATTTGA TGGTGTGACA TCAATGATGT	1680
GTACTCACCA CGGATTATCG GTGGGATTCA CTGGGAACCTA CGAGGAATAC TTTGGACTCG	1740
CAACTGATGT GGATGCTGTT GTGTATCTGA TGCTGGTCAA CGATCTTATT CATGGGCTTT	1800
TCCCAGATGC AATTACCATT GGTGAAGATG TTAGCGGAAT GCCGACATTT TGTGTTCCCG	1860
TTCAAGATGG GGGTGTGGC TTTGACTATC GGCTGCATAT GGCAATTGCT GATAAATGGA	1920
TTGAGTTGCT CAAGAAACGG GATGAGGATT GGAGAGTGGG TGATATTGTT CACACACTGA	1980
CAAATAGAAG ATGGTCGGAA AAGTGTGTTT CATACGCTGA AAGTCATGAT CAAGCTCTAG	2040
TCGGTGATAA AACTATAGCA TTCTGGCTGA TGGACAAGGA TATGTATGAT TTTATGGCTC	2100
TGGATAGACC GTCAACATCA TTAATAGATC GTGGGATAGC ATTACACAAG ATGATTAGGC	2160
TTGTAACAT GGGATTAGGA GGAGAAGGGT ACCTAAATTT CATGGGAAAT GAATTCGGCC	2220
ACCCTGAGTG GATTGATTC CTAGGGCTG AACAAACCT CTCTGATGGC TCAGTAATTC	2280
CCAGAAACCA ATTCAGTTAT GATAAATGCA GACGGAGATT TGACCTGGGA GATGCAGAAT	2340
ATTTAAGATA CCGTGGGTTG CAAGAATTTG ACCGGGCTAT GCAGTATCTT GAAGATAAAT	2400
ATGAGTTTAT GACTTCAGAA CACCAGTTCA TATCACGAAA GGATGAAGGA GATAGGATGA	2460
TTGTATTGA AAAAGGAAAC CTAGTTTTTG TCTTTAATTT TCACCTGGACA AAAGGCTATT	2520
CAGACTATCG CATAGGCTGC CTGAAGCCTG GAAAATACAA GGTGCTTGG GACTCAGATG	2580
ATCCACTTTT TGGTGGCTTC GGGAGAATTG ATCATAATGC CGAATATTC ACCTTTGAAG	2640
GATGGTATGA TGATCGTCT CGTTCAATTA TGGTGTATGC ACCTAGTAGA ACAGCAGTGG	2700
TCTATGCACT AGTAGACAAA GAAGAAGAAG AAGAAGAAGA AGTAGCAGTA GTAGAAGAAG	2760
TAGTAGTAGA AGAAGAATGA ACGAACTTGT GATCGCGTTG AAAGATTTGA ACGCCACATA	2820
GAGCTTCTTG ACGTATCTGG CAATATTGCA TTAGTCTTGG CGGAATTTCA TGTGACAACA	2880
GGTTTGCAAT TCTTTCCACT ATTAGTAGTG CAACGATATA CGCAGAGATG AAGTGCTGAA	2940
CAAAAACATA TGTAATTCG ATGAATTTAT GTCGAATGCT GGGACGATCG AATTCTGCA	3000
GCC	3003

## (2) INFORMATION FOR SEQ ID NO: 13:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2975 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

TTGATGGGCC TTGAAGTCAG CAATTTGACA CTCAGTTAGT TACACTCCTA TCACTTATCA	60
GATCTCTATT TTTTCTCTTA ATTCCAACCA GGGGAATGAA TAAAGGATA GATTGTGAAA	120
AACCCTAAGG AGAGAAGAAG AAAGATGGTG TATATACTCT CTGGAGITCG TTTTCTACT	180
GTTCCATCAG TGTACAAATC TAATGGATTC AGCAGTAATG GTGATCGGAG GAATGCTAAT	240
GTTTCTGTAT TCTTGAAAA GCACCTCTCTT TCACGGAAGA TCTTGGCTGA AAAGTCTTCT	300
TACAATTCGG AATTCCGACC TTCTACAGTT GCAGCATCGG GGAAAGTCCT TGTGCCTGGA	360
ACCCAGAGTG ATAGCTCCTC ATCCTCAACA GACCAATTG AGTTCACTGA GACATCTCCA	420
GAATTCCTCC CAGCATCAAC TGATGTAGAT AGTTCAACA TGGAAACGCG TAGCCAGATT	480
AAAAGTGAAG ACGATGACGT TGAGCCGTCA AGTGATCTTA CAGGAAGTGT TGAAGAGCTG	540
GATTTTGCTT CATCACTACA ACTACAAGAA GGTGGTAAAC TGGAGGAGTC TAAAACATTA	600
AATACTTCTG AAGAGACAAT TATTGATGAA TCTGATAGGA TCAGAGAGAG GGGCATCCCT	660
CCACCTGGAC TTGGTCAGAA GATTATGAA ATAGACCCCC TTTTGACAAA CTATCGTCAA	720
CACCTTGATT ACAGGTATTC ACAGTACAAG AAAGTGAGGG AGGCAATTGA CAAGTATGAG	780
GGTGGTTTGG AAGCTTTTCT CGTGGTTATG AAAAAATGGG TTTCACTCGT AGTGCTACAG	840
GTATCACTTA CCGTGAGTGG GCTCCTGGTG CCCAGTCAGC TGCCCTCATT GGAGATTTC	900
ACAAATGGGA CGCAAATGCT GACATTATGA CTCGGAATGA ATTTGGTGTC TGGGAGATT	960
TTCTGCCAAA TAATGTGGAT GGTTCCTCTG CAATTCCTCA TGGGTCCAGA GTGAAGATAC	1020
GTATGGACAC TCCATCAGGT GTTAAGGATT CCATTCCTGC TTGGATCAAC TACTCTTTAC	1080
AGCTTCTGA TGAAATTCCT TATAATGGAA TATATTATGA TCCACCCGAA GAGGAGAGGT	1140
ATATCTTCCA ACACCCACGG CCAAAGAAAC CAAAGTCGCT GAGAATATAT GAATCTCATA	1200
TTGGAATGAG TAGTCCGGAG CCTAAAATTA ACTCATACGT GAATTTTAGA GATGAAGTTC	1260
TTCTCGCAT AAAAAAGCTT GGTGACAATG CGCTGCGAAT TATGGCTATT CAAGAGCATT	1320
CTTATTATGC TAGTTTTTGGT TATCATGTCA CAAATTTTTT TGCACCAAGC AGCCGTTTTG	1380

GAACGCCGA CGACCTTAAG TCTTCGATTG ATAAAGCTCA TGAGCTAGGA ATTGTTGTTT	1440
TCATGGACAT CGTTCACAGC CATGCATCAA ATAATACTTT AGATGGACTG AACATGTTTG	1500
ACGGCACCGA TAGTTGTTAC TTTCACCTCG GAGCTCGTGG TTATCATTGG ATGTGGGATT	1560
CCGCCTCTTT AACTATGGAA ACTGGGAGGT ACTTAGGTAT CTTCTCTCAA ATGCCAGATG	1620
GTGTTGGAT GAGTTCAAAT TTGATGGATT TAGATTCGAT GGTGTGACAT CAATGATGTA	1680
TACTCACCAC GGATTATCGG TGGGATTAC TGGGAACAC GAGGAATACT TTGGACTCGC	1740
AACTGATGTG GATGCTGTTG TGTATCTGAT GCTGGTCAAC GATCTTATTC ATAGGCTTTT	1800
CCCAGATGCA ATTACCATTG GTGAAGATGT TAGCGGAATG CCGACATTTT GTATTCCCGT	1860
TCAAGATGGG GGTGTTGGCT TTGACTATCG GCTGCATATG GCAATTGCTG ATAAATGGAT	1920
TGAGTTGCTC AAGAAACGGG ATGAGGATTG GAGAGTGGT GATATTGTTT ATACACTGAC	1980
AAATAGAAGA TGGTCGAAA AGTGTGTTTC ATACGCTGAA AGTCATGATC AAGCTCTAGT	2040
CGGTGATAAA ACTATAGCAT TCTGGCTGAT GGACAAGGAT ATGTATGATT TTATGGCTCT	2100
GGATAGACCG CCAACATCAT TAATAGATCG TGGGATAGCA TTGCACAAGA TGATTAGGCT	2160
TGTAACATG GATTAGGAG GAGAAGGTA CCTAAATTTT ATGGGAAATG AATTCGGCCA	2220
CCCTGAGTGG ATTGATTTC CTAGGGCTGA GCCACACCTT TCTGATGGCT CAGTAATTCC	2280
CGGAAACCAA TTCAGTTATG ATAAATGCAG ACGGAGATTT GACCTGGGAG ATGCAGAATA	2340
TTTAAGATAC CATGGGTAC AAGAATTTGA CTGGGCTATG CAGTATCTTG AAGATAATA	2400
TGAGTTTATG ACTTCAGAAC ACCAGTTCAT ATCACGAAAG GATGAAGGAG ATAGGATGAT	2460
TGTATTTGAA AGAGGAAACC TAGTTTTCGT CTTAATTTT CACTGGACAA ATAGCTATTC	2520
AGACTATCGC ATAGGCTGCC TGAAGCCTGG AAAATACAAG GTTGTCTTGG ACTCAGATGA	2580
TCCACTTTTT GGTGGCTTCG GGAGAATTGA TCATAATGCC GAATATTTCA CCTCTGAAGG	2640
ATCGTATGAT GATCGTCCTT GTTCAATTAT GGTGTATGCA CCTAGTAGAA CAGCAGTGGT	2700
CTATGCACTA GTAGACAAAC TAGAAGTAGC AGTAGTAGAA GAACCCATTG AAGAATGAAC	2760
GAACCTTGTA TCGCGTTGAA AGATTGAAAC GTTACTTGGT CATCCACATA GAGCTTCTTG	2820
ACATCAGTCT TGGCGGAATT GCATGTGACA ACAAGGTTTG CAGTCTTTTC CACTATTAGT	2880
AGTCCACCGA TATACGAGA GATGAAGTGC TGAACAAACA TATGTAATAT CGATGAATTT	2940
ATGTCGAATG CTGGGACGAT CGAATTCCTG CAGCC	2975

## (2) INFORMATION FOR SEQ ID NO: 14:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3033 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 145..2790

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

TTGATGGGGC CTTGAACTCA GCAATTTGAC ACTCAGTTAG TTACACTCCT ATCACTTATC	60
AGATCTCTAT TTTTCTCTT AATTCCAACC AAGGAATGAA TAAAGGATA GATTGTAAA	120
AACCCTAAGG AGAGAAGAAG AAAG ATG GTG TAT ACA CTC TCT GGA GTT CGT	171
Met Val Tyr Thr Leu Ser Gly Val Arg	
1 5	
TTT CCT ACT GTT CCA TCA GTG TAC AAA TCT AAT GGA TTC AGC AGT AAT	219
Phe Pro Thr Val Pro Ser Val Tyr Lys Ser Asn Gly Phe Ser Ser Asn	
10 15 20 25	
GGT GAT CGG AGG AAT GCT AAT GTT TCT GTA TTC TTG AAA AAG CAC TCT	267
Gly Asp Arg Arg Asn Ala Asn Val Ser Val Phe Leu Lys Lys His Ser	
30 35 40	
CTT TCA CGG AAG ATC TTG GCT GAA AAG TCT TCT TAC AAT TCC GAA TTC	315
Leu Ser Arg Lys Ile Leu Ala Glu Lys Ser Ser Tyr Asn Ser Glu Phe	
45 50 55	
CGA CCT TCT ACA GTT GCA GCA TCG GGG AAA GTC CTT GTG CCT GGA ACC	363
Arg Pro Ser Thr Val Ala Ala Ser Gly Lys Val Leu Val Pro Gly Thr	
60 65 70	
CAG AGT GAT AGC TCC TCA TCC TCA ACA GAC CAA TTT GAG TTC ACT GAG	411
Gln Ser Asp Ser Ser Ser Ser Ser Thr Asp Gln Phe Glu Phe Thr Glu	
75 80 85	
ACA TCT CCA GAA AAT TCC CCA GCA TCA ACT GAT GTA GAT AGT TCA ACA	459
Thr Ser Pro Glu Asn Ser Pro Ala Ser Thr Asp Val Asp Ser Ser Thr	
90 95 100 105	
ATG GAA CAC GCT AGC CAG ATT AAA ACT GAG AAC GAT GAC GTT GAG CCG	507
Met Glu His Ala Ser Gln Ile Lys Thr Glu Asn Asp Asp Val Glu Pro	
110 115 120	
TCA AGT GAT CTT ACA GGA AGT GTT GAA GAG CTG GAT TTT GCT TCA TCA	555
Ser Ser Asp Leu Thr Gly Ser Val Glu Glu Leu Asp Phe Ala Ser Ser	
125 130 135	



CTA CAA CTA CAA GAA GGT GGT AAA CTG GAG GAG TCT AAA ACA TTA AAT Leu Gln Leu Gln Glu Gly Gly Lys Leu Glu Glu Ser Lys Thr Leu Asn 140 145 150	603
ACT TCT GAA GAG ACA ATT ATT GAT GAA TCT GAT AGG ATC AGA GAG AGG Thr Ser Glu Glu Thr Ile Ile Asp Glu Ser Asp Arg Ile Arg Glu Arg 155 160 165	651
GGC ATC CCT CCA CCT GGA CTT GGT CAG AAG ATT TAT GAA ATA GAC CCC Gly Ile Pro Pro Pro Gly Leu Gly Gln Lys Ile Tyr Glu Ile Asp Pro 170 175 180 185	699
CTT TTG ACA AAC TAT CGT CAA CAC CTT GAT TAC AGG TAT TCA CAG TAC Leu Leu Thr Asn Tyr Arg Gln His Leu Asp Tyr Arg Tyr Ser Gln Tyr 190 195 200	747
AAG AAA CTG AGG GAG GCA ATT GAC AAG TAT GAG GGT GGT TTG GAA GCC Lys Lys Leu Arg Glu Ala Ile Asp Lys Tyr Glu Gly Gly Leu Glu Ala 205 210 215	795
TTT TCT CGT GGT TAT GAA AAA ATG GGT TTC ACT CGT AGT GCT ACA GGT Phe Ser Arg Gly Tyr Glu Lys Met Gly Phe Thr Arg Ser Ala Thr Gly 220 225 230	843
ATC ACT TAC CGT GAG TGG GCT CTT GGT GCC CAG TCA GCT GCC CTC ATT Ile Thr Tyr Arg Glu Trp Ala Leu Gly Ala Gln Ser Ala Ala Leu Ile 235 240 245	891
GGA GAT TTC AAC AAT TGG GAC GCA AAT GCT GAC ATT ATG ACT CGG AAT Gly Asp Phe Asn Asn Trp Asp Ala Asn Ala Asp Ile Met Thr Arg Asn 250 255 260 265	939
GAA TTT GGT GTC TGG GAG ATT TTT CTG CCA AAT AAT GTG GAT GGT TCT Glu Phe Gly Val Trp Glu Ile Phe Leu Pro Asn Asn Val Asp Gly Ser 270 275 280	987
CCT GCA ATT CCT CAT GGG TCC AGA GTG AAG ATA CGT ATG GAC ACT CCA Pro Ala Ile Pro His Gly Ser Arg Val Lys Ile Arg Met Asp Thr Pro 285 290 295	1035
TCA GGT GTT AAG GAT TCC ATT CCT GCT TGG ATC AAC TAC TCT TTA CAG Ser Gly Val Lys Asp Ser Ile Pro Ala Trp Ile Asn Tyr Ser Leu Gln 300 305 310	1083
CTT CCT GAT GAA ATT CCA TAT AAT GGA ATA CAT TAT GAT CCA CCC GAA Leu Pro Asp Glu Ile Pro Tyr Asn Gly Ile His Tyr Asp Pro Pro Glu 315 320 325	1131
GAG GAG AGG TAT ATC TTC CAA CAC CCA CGG CCA AAG AAA CCA AAG TCG Glu Glu Arg Tyr Ile Phe Gln His Pro Arg Pro Lys Lys Pro Lys Ser 330 335 340 345	1179
CTG AGA ATA TAT GAA TCT CAT ATT GGA ATG AGT AGT CCG GAG CCT AAA Leu Arg Ile Tyr Glu Ser His Ile Gly Met Ser Ser Pro Glu Pro Lys 350 355 360	1227

ATT AAC TCA TAC GTG AAT TTT AGA GAT GAA GTT CTT CCT CGC ATA AAA	1275
Ile Asn Ser Tyr Val Asn Phe Arg Asp Glu Val Leu Pro Arg Ile Lys	
365 370 375	
AAG CTT GGG TAC AAT GCG CTG CAA ATT ATG GCT ATT CAA GAG CAT TCT	1323
Lys Leu Gly Tyr Asn Ala Leu Gln Ile Met Ala Ile Gln Glu His Ser	
380 385 390	
TAT TAC GCT AGT TTT GGT TAT CAT GTC ACA AAT TTT TTT GCA CCA AGC	1371
Tyr Tyr Ala Ser Phe Gly Tyr His Val Thr Asn Phe Phe Ala Pro Ser	
395 400 405	
AGC CGT TTT GGA ACG CCC GAC GAC CTT AAG TCT TTG ATT GAT AAA GCT	1419
Ser Arg Phe Gly Thr Pro Asp Asp Leu Lys Ser Leu Ile Asp Lys Ala	
410 415 420 425	
CAT GAG CTA GGA ATT GTT GTT CTC ATG GAC ATT GTT CAC AGC CAT GCA	1467
His Glu Leu Gly Ile Val Val Leu Met Asp Ile Val His Ser His Ala	
430 435 440	
TCA AAT AAT ACT TTA GAT GGA CTG AAC ATG TTT GAC TGC ACC GAT AGT	1515
Ser Asn Asn Thr Leu Asp Gly Leu Asn Met Phe Asp Cys Thr Asp Ser	
445 450 455	
TGT TAC TTT CAC TCT GGA GCT CGT GGT TAT CAT TGG ATG TGG GAT TCC	1563
Cys Tyr Phe His Ser Gly Ala Arg Gly Tyr His Trp Met Trp Asp Ser	
460 465 470	
CGC CTC TTT AAC TAT GGA AAC TGG GAG GTA CTT AGG TAT CTT CTC TCA	1611
Arg Leu Phe Asn Tyr Gly Asn Trp Glu Val Leu Arg Tyr Leu Leu Ser	
475 480 485	
AAT GCG AGA TGG TGG TTG GAT GCG TTC AAA TTT GAT GGA TTT AGA TTT	1659
Asn Ala Arg Trp Trp Leu Asp Ala Phe Lys Phe Asp Gly Phe Arg Phe	
490 495 500 505	
GAT GGT GTG ACA TCA ATG ATG TAT ATT CAC CAC GGA TTA TCG GTG GGA	1707
Asp Gly Val Thr Ser Met Met Tyr Ile His His Gly Leu Ser Val Gly	
510 515 520	
TTC ACT GGG AAC TAC GAG GAA TAC TTT GGA CTC GCA ACT GAT GTG GAT	1755
Phe Thr Gly Asn Tyr Glu Glu Tyr Phe Gly Leu Ala Thr Asp Val Asp	
525 530 535	
GCT GTT GTG TAT CTG ATG CTG GTC AAC GAT CTT ATT CAT GGG CTT TTC	1803
Ala Val Val Tyr Leu Met Leu Val Asn Asp Leu Ile His Gly Leu Phe	
540 545 550	
CCA GAT GCA ATT ACC ATT GGT GAA GAT GTT AGC GGA ATG CCG ACA TTT	1851
Pro Asp Ala Ile Thr Ile Gly Glu Asp Val Ser Gly Met Pro Thr Phe	
555 560 565	
TGT ATT CCC GTC CAA GAG GGG GGT GTT GGC TTT GAC TAT CGG CTG CAT	1899
Cys Ile Pro Val Gln Glu Gly Gly Val Gly Phe Asp Tyr Arg Leu His	
570 575 580 585	

ATG GCA ATT GCT GAT AAA CGG ATT GAG TTG CTC AAG AAA CGG GAT GAG Met Ala Ile Ala Asp Lys Arg Ile Glu Leu Leu Lys Lys Arg Asp Glu 590 595 600	1947
GAT TGG AGA GTG GGT GAT ATT GTT CAT ACA CTG ACA AAT AGA AGA TGG Asp Trp Arg Val Gly Asp Ile Val His Thr Leu Thr Asn Arg Arg Trp 605 610 615	1995
TCG GAA AAG TGT GTT TCA TAC GCT GAA AGT CAT GAT CAA GCT CTA GTC Ser Glu Lys Cys Val Ser Tyr Ala Glu Ser His Asp Gln Ala Leu Val 620 625 630	2043
GGT GAT AAA ACT ATA GCA TTC TGG CTG ATG GAC AAG GAT ATG TAT GAT Gly Asp Lys Thr Ile Ala Phe Trp Leu Met Asp Asp Met Tyr Asp 635 640 645	2091
TTT ATG GCT CTG GAT AGA CCG TCA ACA TCA TTA ATA GAT CGT GGG ATA Phe Met Ala Leu Asp Arg Pro Ser Thr Ser Leu Ile Asp Arg Gly Ile 650 655 660 665	2139
GCA TTG CAC AAG ATG ATT AGG CTT GTA ACT ATG GGA TTA GGA GGA GAA Ala Leu His Lys Met Ile Arg Leu Val Thr Met Gly Leu Gly Gly Glu 670 675 680	2187
GGG TAC CTA AAT TTC ATG GGA AAT GAA TTC GGC CAC CCT GAG TGG ATT Gly Tyr Leu Asn Phe Met Gly Asn Glu Phe Gly His Pro Glu Trp Ile 685 690 695	2235
GAT TTC CCT AGG GCT GAA CAA CAC CTC TCT GAT GGC TCA GTA ATC CCC Asp Phe Pro Arg Ala Glu Gln His Leu Ser Asp Gly Ser Val Ile Pro 700 705 710	2283
GGA AAC CAA TTC AGT TAT GAT AAA TGC AGA CGG AGA TTT GAC CTG GGA Gly Asn Gln Phe Ser Tyr Asp Lys Cys Arg Arg Arg Phe Asp Leu Gly 715 720 725	2331
GAT GCA GAA TAT TTA AGA TAC CGT GGG TTG CAA GAA TTT GAC CGG CCT Asp Ala Glu Tyr Leu Arg Tyr Arg Gly Leu Gln Glu Phe Asp Arg Pro 730 735 740 745	2379
ATG CAG TAT CTT GAA GAT AAA TAT GAG TTT ATG ACT TCA GAA CAC CAG Met Gln Tyr Leu Glu Asp Lys Tyr Glu Phe Met Thr Ser Glu His Gln 750 755 760	2427
TTC ATA TCA CGA AAG GAT GAA GGA GAT AGG ATG ATT GTA TTT GAA AAA Phe Ile Ser Arg Lys Asp Glu Gly Asp Arg Met Ile Val Phe Glu Lys 765 770 775	2475
GGA AAC CTA GTT TTT GTC TTT AAT TTT CAC TGG ACA AAA AGC TAT TCA Gly Asn Leu Val Phe Val Phe Asn Phe His Trp Thr Lys Ser Tyr Ser 780 785 790	2523
GAC TAT CGC ATA GCC TGC CTG AAG CCT GGA AAA TAC AAG GTT GCC TTG Asp Tyr Arg Ile Ala Cys Leu Lys Pro Gly Lys Tyr Lys Val Ala Leu 795 800 805	2571

46

GAC TCA GAT GAT CCA CTT TTT GGT GGC TTC GGG AGA ATT GAT CAT AAT 2619  
 Asp Ser Asp Asp Pro Leu Phe Gly Gly Phe Gly Arg Ile Asp His Asn 825  
 810 815 820  
 GCC GAA TAT TTC ACC TTT GAA GGA TGG TAT GAT GAT CGT CCT CGT TCA 2667  
 Ala Glu Tyr Phe Thr Phe Glu Gly Trp Tyr Asp Asp Arg Pro Arg Ser 840  
 830 835  
 ATT ATG GTG TAT GCA CCT TGT AAA ACA GCA GTG GTC TAT GCA CTA GTA 2715  
 Ile Met Val Tyr Ala Pro Cys Lys Thr Ala Val Val Tyr Ala Leu Val 855  
 845 850  
 GAC AAA GAA GAA GAA GAA GAA GAA GAA GAA GAA GAA GTA GCA GCA 2763  
 Asp Lys Glu Glu Glu Glu Glu Glu Glu Glu Glu Val Ala Ala 870  
 860 865  
 GTA GAA GAA GTA GTA GTA GAA GAA GAA TGAACGAACT TGTGATCGCG 2810  
 Val Glu Glu Val Val Val Glu Glu Glu 880  
 875 880  
 TTGAAAGATT TGAACGCTAC ATAGAGCTTC TTGACGTATC TGGCAATATT GCATCAGTCT 2870  
 TGCGCGAATT TCATGTGACA CAAGGTTTGC AATTCTTTCC ACTATTAGTA GTGCAACGAT 2930  
 ATACGCAGAG ATGAAGTGCT GAACAAACAT ATGTAAATC GATGAATTTA TGTCGAATGC 2990  
 TGGGACGATC GAATTCCTGC AGGCCGGGGG ACCCCTTAGT TCT 3033

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 882 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Met Val Tyr Thr Leu Ser Gly Val Arg Phe Pro Thr Val Pro Ser Val  
 1 5 10 15  
 Tyr Lys Ser Asn Gly Phe Ser Ser Asn Gly Asp Arg Arg Asn Ala Asn  
 20 25 30  
 Val Ser Val Phe Leu Lys Lys His Ser Leu Ser Arg Lys Ile Leu Ala  
 35 40 45  
 Glu Lys Ser Ser Tyr Asn Ser Glu Phe Arg Pro Ser Thr Val Ala Ala  
 50 55 60  
 Ser Gly Lys Val Leu Val Pro Gly Thr Gln Ser Asp Ser Ser Ser Ser  
 65 70 75 80  
 Ser Thr Asp Gln Phe Glu Phe Thr Glu Thr Ser Pro Glu Asn Ser Pro  
 85 90 95

Ala Ser Thr Asp Val Asp Ser Ser Thr Met Glu His Ala Ser Gln Ile  
 100 105 110  
 Lys Thr Glu Asn Asp Asp Val Glu Pro Ser Ser Asp Leu Thr Gly Ser  
 115 120 125  
 Val Glu Glu Leu Asp Phe Ala Ser Ser Leu Gln Leu Gln Glu Gly Gly  
 130 135 140  
 Lys Leu Glu Glu Ser Lys Thr Leu Asn Thr Ser Glu Glu Thr Ile Ile  
 145 150 155 160  
 Asp Glu Ser Asp Arg Ile Arg Glu Arg Gly Ile Pro Pro Pro Gly Leu  
 165 170 175  
 Gly Gln Lys Ile Tyr Glu Ile Asp Pro Leu Leu Thr Asn Tyr Arg Gln  
 180 185 190  
 His Leu Asp Tyr Arg Tyr Ser Gln Tyr Lys Lys Leu Arg Glu Ala Ile  
 195 200 205  
 Asp Lys Tyr Glu Gly Gly Leu Glu Ala Phe Ser Arg Gly Tyr Glu Lys  
 210 215 220  
 Met Gly Phe Thr Arg Ser Ala Thr Gly Ile Thr Tyr Arg Glu Trp Ala  
 225 230 235 240  
 Leu Gly Ala Gln Ser Ala Ala Leu Ile Gly Asp Phe Asn Asn Trp Asp  
 245 250 255  
 Ala Asn Ala Asp Ile Met Thr Arg Asn Glu Phe Gly Val Trp Glu Ile  
 260 265 270  
 Phe Leu Pro Asn Asn Val Asp Gly Ser Pro Ala Ile Pro His Gly Ser  
 275 280 285  
 Arg Val Lys Ile Arg Met Asp Thr Pro Ser Gly Val Lys Asp Ser Ile  
 290 295 300  
 Pro Ala Trp Ile Asn Tyr Ser Leu Gln Leu Pro Asp Glu Ile Pro Tyr  
 305 310 315 320  
 Asn Gly Ile His Tyr Asp Pro Pro Glu Glu Glu Arg Tyr Ile Phe Gln  
 325 330 335  
 His Pro Arg Pro Lys Lys Pro Lys Ser Leu Arg Ile Tyr Glu Ser His  
 340 345 350  
 Ile Gly Met Ser Ser Pro Glu Pro Lys Ile Asn Ser Tyr Val Asn Phe  
 355 360 365  
 Arg Asp Glu Val Leu Pro Arg Ile Lys Lys Leu Gly Tyr Asn Ala Leu  
 370 375 380  
 Gln Ile Met Ala Ile Gln Glu His Ser Tyr Tyr Ala Ser Phe Gly Tyr  
 385 390 395 400

His Val Thr Asn Phe Phe Ala Pro Ser Ser Arg Phe Gly Thr Pro Asp  
 405 410 415  
 Asp Leu Lys Ser Leu Ile Asp Lys Ala His Glu Leu Gly Ile Val Val  
 420 425 430  
 Leu Met Asp Ile Val His Ser His Ala Ser Asn Asn Thr Leu Asp Gly  
 435 440 445  
 Leu Asn Met Phe Asp Cys Thr Asp Ser Cys Tyr Phe His Ser Gly Ala  
 450 455 460  
 Arg Gly Tyr His Trp Met Trp Asp Ser Arg Leu Phe Asn Tyr Gly Asn  
 465 470 475 480  
 Trp Glu Val Leu Arg Tyr Leu Leu Ser Asn Ala Arg Trp Trp Leu Asp  
 485 490 495  
 Ala Phe Lys Phe Asp Gly Phe Arg Phe Asp Gly Val Thr Ser Met Met  
 500 505 510  
 Tyr Ile His His Gly Leu Ser Val Gly Phe Thr Gly Asn Tyr Glu Glu  
 515 520 525  
 Tyr Phe Gly Leu Ala Thr Asp Val Asp Ala Val Val Tyr Leu Met Leu  
 530 535 540  
 Val Asn Asp Leu Ile His Gly Leu Phe Pro Asp Ala Ile Thr Ile Gly  
 545 550 555 560  
 Glu Asp Val Ser Gly Met Pro Thr Phe Cys Ile Pro Val Gln Glu Gly  
 565 570 575  
 Gly Val Gly Phe Asp Tyr Arg Leu His Met Ala Ile Ala Asp Lys Arg  
 580 585 590  
 Ile Glu Leu Leu Lys Lys Arg Asp Glu Asp Trp Arg Val Gly Asp Ile  
 595 600 605  
 Val His Thr Leu Thr Asn Arg Arg Trp Ser Glu Lys Cys Val Ser Tyr  
 610 615 620  
 Ala Glu Ser His Asp Gln Ala Leu Val Gly Asp Lys Thr Ile Ala Phe  
 625 630 635 640  
 Trp Leu Met Asp Lys Asp Met Tyr Asp Phe Met Ala Leu Asp Arg Pro  
 645 650 655  
 Ser Thr Ser Leu Ile Asp Arg Gly Ile Ala Leu His Lys Met Ile Arg  
 660 665 670  
 Leu Val Thr Met Gly Leu Gly Gly Glu Gly Tyr Leu Asn Phe Met Gly  
 675 680 685  
 Asn Glu Phe Gly His Pro Glu Trp Ile Asp Phe Pro Arg Ala Glu Gln  
 690 695 700

His Leu Ser Asp Gly Ser Val Ile Pro Gly Asn Gln Phe Ser Tyr Asp  
 705 710 715 720  
 Lys Cys Arg Arg Arg Phe Asp Leu Gly Asp Ala Glu Tyr Leu Arg Tyr  
 725 730 735  
 Arg Gly Leu Gln Glu Phe Asp Arg Pro Met Gln Tyr Leu Glu Asp Lys  
 740 745 750  
 Tyr Glu Phe Met Thr Ser Glu His Gln Phe Ile Ser Arg Lys Asp Glu  
 755 760 765  
 Gly Asp Arg Met Ile Val Phe Glu Lys Gly Asn Leu Val Phe Val Phe  
 770 775 780  
 Asn Phe His Trp Thr Lys Ser Tyr Ser Asp Tyr Arg Ile Ala Cys Leu  
 785 790 795 800  
 Lys Pro Gly Lys Tyr Lys Val Ala Leu Asp Ser Asp Asp Pro Leu Phe  
 805 810 815  
 Gly Gly Phe Gly Arg Ile Asp His Asn Ala Glu Tyr Phe Thr Phe Glu  
 820 825 830  
 Gly Trp Tyr Asp Asp Arg Pro Arg Ser Ile Met Val Tyr Ala Pro Cys  
 835 840 845  
 Lys Thr Ala Val Val Tyr Ala Leu Val Asp Lys Glu Glu Glu Glu Glu  
 850 855 860  
 Glu Glu Glu Glu Glu Glu Val Ala Ala Val Glu Glu Val Val Val Glu  
 865 870 875 880  
 Glu Glu

## (2) INFORMATION FOR SEQ ID NO: 16:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 2576 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

TCATTAAAGA GGAGAAATTA ACTATGAGAG GATCTCACCA TCACCATCAC CATGGGATCT	60
TGGTGTA AAA GTCTTCTTAC AATTCGGAAT TCCGACCTTC TACAGTTGCA GCATCGGGGA	120
AAGTCCTTGT GCCTGGAACC CAGAGTGATA GCTCCTCATC CTCACAAAC CAATTTGAGT	180
TCACTGAGAC ATCTCCAGAA AATCCCCAG CATCAACTGA TGATAGATAGT TCAACAATGG	240
AACACGCTAG CCAGATTAAA ACTGAGAACG ATGACGTTGA GCCGTCAAGT GATCTTACAG	300

GAAGTGTGA AGAGCTGGAT TTTGCTTCAT CACTACAAC	360
AGGAGTCTAA AACATTAAT ACTTCTGAAG AGACAATTAT	420
GAGAGAGGGG CATCCCTCCA CCTGGACTTG GTCAGAAGAT	480
TGACAACTA TCGTCAACAC CTTGATTACA GGTATTACA	540
CAATTGACAA GTATGAGGGT GGTGGGAAG CTTTTCTCG	600
TCACTCGTAG TGCTACAGGT ATCACTTACC GTGAGTGGGC	660
CCCTCATTGG AGATTCAAC AATTGGGACG CAAATGCTGA	720
TTGGTGTCTG GGAGATTTTT CTGCCAAATA ATGGGATGG	780
GGTCCAGAGT GAAGATACGT ATGGACACTC CATCAGGTGT	840
GGATCACTA CTCTACAGCT TCCTGATGAA ATTCCATATA	900
CCCAGAGAGG AGAGGTATAT CTTCCAACAC CCACGGCCAA	960
ATATATGAAT CTCATATTGG AATGAGTAGT CCGGAGCCTA	1020
TTTAGAGATG AAGTCTCTCC TCGCATAAAA AAGCTTGGT	1080
GCTATTCAAG AGCATTCTTA TTATGCTAGT TTTGGTTATC	1140
CCAAGCAGCC GTTTTGAAC GCCCGACGAC CTTAAGTCTT	1200
CTAGGAATTG TTGTTCTCAT GGACATTGTT CACAGCCATG	1260
GGACTGAACA TGTGTGACGG CACCGATAGT TGTACTTTC	1320
CATTGGATGT GGGATTCCCG CCTTTTAAAC TATGGAACT	1380
CTCTCAAATG CGAGATGGTG GTTGGATGAG TTCAAATTTG	1440
GTGACATCAA TGATGTATAC TCACCACGGA TTATCGGTGG	1500
GAATACTTTG GACTCGCAAC TGATGTGGAT GCTGTTGTGT	1560
CTTATTCATG GGCTTTTCCC AGATGCAATT ACCATTGGTG	1620
ACATTTTGTA TTCCCGTTCA AGATGGGGGT GTTGGCTTTG	1680
ATTGCTGATA AATGGATTGA GTTGCTCAAG AACGGGATG	1740
ATTGTTTATA CACTGACAAA TAGAAGATGG TCGGAAAAGT	1800
CATGATCAAG CTCTAGTCGG TGATAAACT ATAGCATTCT	1860
TATGATTTTA TGGCTCTGGA TAGACGCCA ACATCATTAA	1920
CACAAGATGA TTAGGCTTGT AACTATGGGA TTAGGAGGAG	1980



GGAAATGAAT TCGGCCACCC TGAGTGGATT GATTTCCCTA GGGCTGAACA ACACCTCTCT	2040
GATGACTCAG TAATTCCTCGG AAACCAATTC AGTTATGATA AATGCAGACG GAGATTTGAC	2100
CTGGGAGATG CAGAATATTT AAGATACCGT GGGTTGCAAG AATTTGACCG GGCTATGCAG	2160
TATCTTGAAG ATAAATATGA GTTTATGACT TCAGAACACC AGTTCATATC ACGAAAGGAT	2220
GAAGGAGATA GGATGATTGT ATTTGAAAAA GGAAACCTAG TTTTGTCTT TAATTTTCAC	2280
TGGACAAAAA GCTATTCAGA CTATCGCATA GGCTGCCTGA AGCCTGGAAA ATACAAGGTT	2340
GCCTTGGACT CAGATGATCC ACTTTTTGGT GGCTTCGGA GAATTGATCA TAATGCCGAA	2400
TATTCACCT TTGAAGGATG GTATGATGAT CGTCTCGTT CAATTATGTT GTATGCACCT	2460
TGTAGAACAG CAGTGGTCTA TGCCTAGTA GACAAAGAAG AAGAAGAAGA AGAAGAAGAA	2520
GAAGAAGTAG CAGTAGTAGA AGAAGTAGTA GTAGAAGAAG AATGAACGAA CTTGTG	2576

## (2) INFORMATION FOR SEQ ID NO: 17:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2529 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GGATGCTAAT GTTCTGTAT TCTTGAAAAA GCACTCTCTT TCACGGAAGA TCTTGGCTGA	60
AAAGTCTTCT TACAATCCG AATCCCGACC TTCTACAGTT GCAGCATCGG GGAAAGTCCT	120
TGTGCTCGGA AYCCAGAGTG ATAGCTCCTC ATCCTCAACA GACCAATTGG AGTTCACTGA	180
GACATCTCCA GAAAATTCCC CAGCATCAAC TGATGTAGAT AGTTCAACAA TGGAACACGC	240
TAGCCGATT AAAACTGAGA ACGATGACGT TGAGCCGTCA AGTGATCTTA CAGGAAGTGT	300
TGAAGAGCTG GATTTTGCTT CATCACTACA ACTACAAGAA GGTGGTAAAC TGGAGGAGTC	360
TAAACATTA AATACTTCTG AAGAGACAAT TATTGATGAA TCTGATAGGA TCAGAGAGAG	420
GGGCATCCCT CCACCTGGAC TTGGTCAGAA GATTATGAA ATAGACCCCC TTTTGACAAA	480
CTATCGTCAA CACCTTGATT ACAGGTATTC ACAGTACAAG AACTGAGGG AGGCAATTGA	540
CAAGTATGAG GGTGGTTTGG AAGCTTTTTC TCGTGGTTAT GAAAAAATGG GTTTCACCTG	600
TAGTGCTACA GGTATCACTT ACCGTGAGTG GGCTCCTGGT GCCCAGTCAG CTGCCCTCAT	660
TGGAGATTTT AACAAATGGG ACGCAATATG TGACATTATG ACTCGGAATG AATTTGGTGT	720
CTGGGAGATT TTTCTGCCAA ATAATGTGGA TGGTTCTCCT GCAATTCCTC ATGGGTCCAG	780

AGTGAAGATA CGYATGGACA CTCCATCAGG TGTTAAGGAT TCCATTCCCTG CTTGGATCAA	840
CTACTCTTTA CAGCTTCCTG ATGAAATTCC ATATAATGGA ATATATTATG ATCCACCCGA	900
AGAGGAGAGG TATRTCTTCC AACACCCACG GCCAAAGAAA CCAAAGTCGC TGAGAATATA	960
TGAATCTCAT ATTGGAATGA GTAGTCCGGA GCCTAAAATT AACTCATACG TGAATTTTAG	1020
AGATGAAGTT CTTCTCGCA TAAAAAASCT TGGGTACAAT GCGGTGCAAA TTATGGCTAT	1080
TCAAGAGCAT TCTTATTATG CTAGTTTTGG TTATCATGTC ACAAATTTTT TTGCACCAAG	1140
CAGCCGTTTT GGAACGCCCG ACGACCTTAA GTCTTTGATT GATAAAGCTC ATGAGCTAGG	1200
AATTGTGTGT CTCATGGACA TTGTTCACAG CCATGCATCA AATAATACTT TAGATGGACT	1260
GAACATGTTT GACGGCACAG ATAGTTGTTA CTTTCACTCT GGAGCTCGTG GTTATCATTG	1320
GATGTGGGAT TCCCCTCTCT TTAATATGG AAAGTGGGAG GTACTTAGGT ATCTTCTCTC	1380
AAATGCGAGA TGGTGGTTGG ATGAGTTCAA ATTTGATGGA TTTAGATTTG ATGGTGTGAC	1440
ATCAATGATG TATACTCACC ACGGATTATC GGTGGGATTC ACTGGGAACT ACGAGGAATA	1500
CTTTGGACTC GCAACTGATG TGGATGCTGT TGTGTATCTG ATGCTGGTCA ACGATCTTAT	1560
TCACGGGCTT TTCCAGATG CAATTACCAT TGGTGAAGAT GTTAGCGGAA TGCCGACATT	1620
TTGTATTTCC GTTCAAGATG GGGGTGTTGG CTTTGACTAT CGGCTGCATA TGGCAATTGC	1680
TGATAAATGG ATTGAGTTGC TCAAGAAACG GGATGAGGAT TGGAGAGTGG GTGATATTGT	1740
TCATACACTG ACAAATAGAA GATGGTCGGA AAAGTGTTGT TCATMCGCTG AAAGTCATGA	1800
TCAAGCTCTA GTCGGTGATA AAATATAGC ATYCTGGCTG ATGGACAAGG ATATGTATGA	1860
TTTTATGGCT CTGGATAGAC CGYCAACAYC ATTAATAGAT CGTGGGATAG CATTGCACAA	1920
GATGATTAGG CTGTAACTA TGGGATTAGG AGGAGAAGGG TACCTAAATT TCATGGGAAA	1980
TGAATTCGGC CACCTGAGT GGATTGATTT CCCTAGGGCT GARCAACACC TCTCTGATGG	2040
CTCAGTAATT CCCGAAACC AATTCAGTTA TGATAAATGC AGACGGAGAT TTGACCTGGG	2100
AGATGCAGAA TATTTAAGAT ACCATGGGTT GCAAGAATTT GACCGGGCTA TGCAGTATCT	2160
TGAAGATAAA TATGAGTTTA TGACTTCAGA ACACCAGTTC ATATCACGAA AGGATGAAGG	2220
AGATAGGATG ATTGTATTTG AAARAGGAAA CCTAGTTTTT GTCTTTAATT TTCACTGGAC	2280
AAATAGCTAT TCAGACTATC GCATAGGCTG CCTGAAGCCT GGAAAATACA AGGTTGGCTT	2340
GGACTCAGAT GATCCACTTT TTGGTGGCTT CGGGAGAATT GATCATAATG CCGAATATTT	2400
CACCTCTGAA GGATCGTATG ATGATCGTCC TCGTTCAATT ATGGTGTATG CACCTAGTAG	2460

AACAGCAGTG GTCTATGCAC TAGTAGACAA ANTAGAAGNA GAAGAAGAAG AAGAANCCGN 2520  
 NGAAGAATT 2529

## (2) INFORMATION FOR SEQ ID NO: 18:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3231 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GATTTAATAC GACTCACTAT AGGGATTTTT TTTTTTTTT TTTTAAAAAC CTCCTCCACT 60  
 CAGTCTTGGG ATCTCTCTCT CTCTTCACGC TTCTCTTGGG GCCTTGAAC CAGCAATTTG 120  
 ACACTCAGTT AGTTACATC CTATCACTCA TCAGATCTCT ATTTTTTCTC TTAATTCCAA 180  
 CCAAGGAATG AATTTAAAGA TTAGATTGA AGGAGAGAAG AAGAAAGATG GTGTATACAC 240  
 TCTCTGGAGT TCGTTTTCT ACTGTTCCAT CAGTGTAACA ATCTAATGGA TTCAGCAGTA 300  
 ATGGTGATCG GAGGAATGCT AATGTTTCTG TATTCTTGAA AAAGCACTCT CTTTCACGGA 360  
 AGATCTTGGC TGAAAAGTCT TCTTACGATT CCGAATCCCG ACCTTCTACA GTTGACAGCAT 420  
 CGGGGAAAGT CTTGTACCT GGAATCCAGA GTGATAGCTC CTCATCCTCA ACAGACCAAT 480  
 TTGAGTTTAC TGAGACAGCT CCAGAAAATT CCCAGCATC AACTGATGTG GATAGTTCAA 540  
 CAATGGAACA CGTAGCCAG ATTTAACTG AGAACGATGA CGTTGAGCCG TCAAGTGATC 600  
 TTACAGGAAG TGTGAAGAG TTGGATTTTG CTTCACTACT ACAACTACAA GAAGGTGGTA 660  
 AACTGGAGGA GTCTAAAACA TTAATACTT CTGAAGAGAC AATTATTGAT GAATCTGATA 720  
 GGATCAGAGA GAGGGGCATC CCTCCACCTG GACTTGGTCA GAAGATTAT GAAATAGACC 780  
 CCCTTTTGAC AACTATCGT CAACACCTTG ATTACAGGTA TTCACAGTAC AAGAAAATGA 840  
 GGGAGGCAAT TGACAAGTAT GAGGGTGGT TGAAGCTTT TTCTCGTGGT TATGAAAAAA 900  
 TGGGTTTAC TCGTAGTCT ACAGGTATCA CTTACCGTGA GTGGGCTCCT GGTGCCCACT 960  
 CAGCTGCTCT CATTGGAGAT TTCAACAATT GGGACGCAA TGCTGACATT ATGACTCGGA 1020  
 ATGAATTTGG TGCTGGGAG ATTTTCTGC CAAATAATGT GGATGGTTCT CCGCAATTC 1080  
 CTCATGGGTC CAGAGTGAAG ATACGCATGG ACACCTCATC AGGTGTTAAG GATTCCATTC 1140  
 CTGCTTGGAT CAACTACTCT TTACAGCTTC CTGATGAAAT TCCATATAAT GGAATATATT 1200  
 ATGATCCACC CGAAGAGGAG AGGTATGTCT TCCACACCC ACGGCCAAAG AAACCAAAGT 1260

CGCTGAGAAT ATATGAATCT CATATTGGAA TGAGTAGTCC GGAGCCTAAA ATTAACATCAT	1320
ACGTGAATTT TAGAGATGAA GTTCTTCTC GCATAAAAAA CCTTGGGTAC AATGCGGTGC	1380
AAATTATGGC TATTCAAGAG CATTCTTATT ATGCTAGTTT TGGTTATCAT GTCACAAATT	1440
TTTTTGACC AAGCAGCCGT TTTGGAACGC CCGACGACCT TAAGTCTTTG ATTGATAAAG	1500
CTCATGAGCT AGGAATTGTT GTTCTCATGG ACATTGTTCA CAGCCATGCA TCAAATAATA	1560
CTTTAGATGG ACTGAACATG TTTGACGGCA CAGATAGTTG TTACTTTTAC TCTGGAGCTC	1620
GTGGTTATCA TTGGATGTGG GATTCCCGCC TCTTTAACTA TGGAACTGG GAGGTACTTA	1680
GGTATCTTCT CTCAAATGCG AGATGGTGGT TGGATGAGTG CAAATTTGRT GGATTAGAT	1740
TTGATGGTGT GACATCAATG ATGTATACTC ACCACGGATT ATCGGTGGGA TTCACTGGGA	1800
ACTACGAGGA ATACTTTGGA CTCGCAACTG ATGTRGATGC TGCCGTGTAT CTGATGCTGG	1860
CCAACGATCT TATTCATGGG CTTTTCCAG ATGCAATTAC CATTGGTGAA GATGTTAGCG	1920
GAATGCCGAC ATTTTGTATT CCCGTTCAAG ATGGGGGTGT TGGCTTTGAC TATCGGCTGC	1980
ATATGGCAAT TGCTGATAAA TGGATTGAGT TGCTCAAGAA ACGGGATGAG GATTGGAGAG	2040
TGGGTGATAT TGTTCATACA CTGACAAATA GAAGATGGTC GGAAAAGTGT GTTTCATACG	2100
CTGAAAGTCA TGATCAAGCT CTAGTCGGTG ATAAACTAT AGCATTCTGG CTGATGGACA	2160
AGGATATGTA TGATTTTATG GCTTTGGATA GACCGTCAAC ATCATTAAATA GATCGTGGGA	2220
TAGCATTGCA CAAGATGATT AGGCTTGTA CTATGGGATT AGGAGGAGAA GGGTACCTAA	2280
ATTTTCATGGG AAATGAATTC GGCCACCCTG AGTGGATTGA TTTCCCTAGG GCTGAACAAC	2340
ACCTCTCTGA TGGCTCAGTA ATTCCCGGAA ACCAATTCAG TTATGATAAA TGCAGACGGA	2400
GATTTGACCT GGGAGATGCA GAATATTTAA GATACCGTGG GTTGCAAGAA TTTGACCGGG	2460
CTATGCAGTA TCTTGAAGAT AAATATGAGT TTATGACTTC AGAACACCAG TTCATATCAC	2520
GAAAGGATGA AGGAGATAGG ATGATTGTAT TTGAAAAAGG AAACCTAGTT TTTGTCTTTA	2580
ATTTTCACTG GACAAAAAGC TATTCAGACT ATCGCATAGG CTGGCTGAAG CCTGGAAAAAT	2640
ACAAGGTTGC CTTGACTCA GATGATCCAC TTTTGGTGG CTTCGGGAGA ATTGATCATA	2700
ATGCCGAATG TTTACCTTT GAAGGATGGT ATGATGATCG TCCTCGTTCA ATTATGGTGT	2760
ATGCACCTAG TAGAACAGCA GTGGTCTATG CACTAGTAGA CAAAGAAGAA GAGAAGAAG	2820
AAGTAGCAGT AGTAGAAGAA GTAGTAGTAG AAGAAGAATG AACGAACTTG TGATCGCGTT	2880
GAAAGATTG AACGCTACAT AGAGCTTCTT GACGTATCTG GCAATATTGC ATCAGTCTTG	2940

CGGGAATTC ATGTGACAAA AGGTTTGCAA TTCTTTCCAC TATTAGTAGT GCAACGATAT	3000
ACGCAGAGAT GAAGTGCTGA ACAACATAT GTAAAAATCGA TGAATTTATG TCGAATGCTG	3060
GGACGGGCTT CAGCAGGTTT TGCTTAGTGA GTTCTGTAAA TTGTCATCTC TTTANATGTA	3120
CAGCCCACTA GAAATCAATT ATGTGAGACC TAAAAACAA TAACCATAAA ATGGAAATAG	3180
TGCTGATCTA ATGATGTTTT AANCCNNNA AAAAAAAAAA AAAAACTCGA G	3231

## (2) INFORMATION FOR SEQ ID NO: 19:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2578 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TCATTAAAGA GGAGAAATTA ACTATGAGAG GATCTCACCA TCACCATCAC CATGGGATCT	60
TGGCTGAAAA GTCTTCTTAC AATTCGAAT TCCGACCTTC TACAGTTGCA GCATCGGGGA	120
AAGTCTTGT GCCTGGAACC CAGAGTGATA GCTCCTCATC CTCAACAAAC CAATTTGAGT	180
TCACTGAGAC ATCTCCAGAA AATTCCTCAG CATCAACTGA TGAGATAGT TCAACAATGG	240
AACACGCTAG CCAGATTAAT ACTGAGAACG ATGACGTTGA GCCGTCAAGT GATCTTACAG	300
GAAGTGTTGA AGAGCTGGAT TTTGCTTCAT CACTACAAC ACAAGAAGGT GGTAACTGG	360
AGGAGTCTAA AACATTAAAT ACTTCTGAAG AGACAATTAT TGATGAATCT GATAGGATCA	420
GAGAGAGGGG CATCCCTCCA CCTGGACTTG GTCAGAAGAT TTATGAAATA GACCCCTTT	480
TGACAAACTA TCGTCAACAC CTTGATTACA GGTATTCACA GTACAAGAAA CTGAGGGAGG	540
CAATTGACAA GTATGAGGGT GGTTTGGAAG CTTTTTCTCG TGTTATGAA AAAATGGGT	600
TCACTCGTAG TGCTACAGGT ATCACTTACC GTGAGTGGGC TCCTGGTGCC CAGTCAGCTG	660
CCCTCATTGG AGATTCAAC AATTGGGACG CAAATGCTGA CATTATGACT CGGAATGAAT	720
TTGGTGTCTG GGAGATTTTT CTGCCAAATA ATGTGGATGG TTCTCTGCA ATTCCTCATG	780
GGTCCAGAGT GAAGATACGT ATGGACACTC CATCAGGTGT TAAGGATTCC ATTCCTGCTT	840
GGATCAACTA CTCTTCACAG CTTCTGATG AAATCCATA TAATGGAATA TATTATGATC	900
CACCCGAAGA GGAGAGGTAT ATCTTCAAC ACCCACGGCC AAAGAAACCA AAGTCGCTGA	960
GAATATATGA ATCTCATATT GGAATGAGTA GTCCGGAGCC TAAATTAAC TCATACGTGA	1020
ATTTTAGAGA TGAAGTTCTT CCTCGCATAA AAAAGCTTGG GTACAATGCG GTGCAAATTA	1080

TGGCTATTCA AGAGCATTCT TATTATGCTA GTTTTGGTTA TCATGTCACA AATTTTTTTG	1140
CACCAAGCAG CCGTTTTGGA ACGCCCGACG ACCTTAAGTC TTTGATTGAT AAAGCTCATG	1200
AGCTAGGAAT TGTGTTCTC ATGGACATTG TTCACAGCCA TGCATCAAA AATACTTTAG	1260
ATGGA CTGAA CATGTTTGAC GGCACCGATA GTTGTTACTT TCACTCTGGA GCTCGTGGTT	1320
ATCATGGGAT GTGGGATTCC CGCCTTTTTA ACTATGGAAA CTGGGAGGTA CTTAGGTATC	1380
TTCTCTCAAA TGCAGATGG TGGTTGGATG AGTTCAAATT TGATGGATTT AGATTTGATG	1440
GTGTGACATC AATGATGTAT ACTCACCACG GATTATCGGT GGGATTCAC TGGAACTACG	1500
AGGAATACTT TGGACTCGCA ACTGATGTGG ATGCTGTTGT GTATCTGATG CTGGTCAACG	1560
ATCTTATTCA TGGGCTTTTC CCAGATGCAA TTACCATTGG TGAAGATGTT AGCGGAATGC	1620
CGACATTTTG TATTCCTGTT CAAGATGGGG GTGTTGGCTT TGA CTGATCTG CTGCATATGG	1680
CAATTGCTGA TAAATGGATT GAGTTGCTCA AGAAACGGGA TGAGGATTGG AGAGTGGGTG	1740
ATATTGTTCA TACTGACA AATAGAAGAT GGTCTGAAAA GTGTGTTTCA TACGTGAAA	1800
GTCATGATCA AGCTCTAGTC GGTGATAAAA CTATAGCATT CTGGCTGATG GACAAGGATA	1860
TGTATGATTT TATGGCTCTG GATAGACCGC CAACATCATT AATAGATCGT GGGATAGCAT	1920
TGCACAAGAT GATTAGGCTT GTAACATG GATTAGGAGG AGAAGGGTAC CTAAATTTCA	1980
TGGGAAATGA ATTCGGCCAC CCTGAGTGGA TTGATTTCCC TAGGGCTGAA CAACACCTCT	2040
CTGATGACTC AGTAATTTCC GGAACCAAT TCAGTTATGA TAAATGCAGA CGGAGATTTG	2100
ACCTGGGAGA TGCAGAATAT TTAAGATACC GTGGGTTGCA AGAATTTGAC CGGGCTATGC	2160
AGTATCTTGA AGATAAATAT GAGTTTATGA CTTGAGAA CAAGTTTCTA TCACGAAAGG	2220
ATGAAGGAGA TAGGATGATT GTATTTGAAA AAGGAAACCT AGTTTTTGT TTTAATTTTC	2280
ACTGGACAAA AAGCTATTCA GACTATCGCA TAGGCTGCCT GAAGCCTGGA AAATACAAGG	2340
TTGCCTTGGA CTCAGATGAT CCACTTTTTG GTGGCTTCGG GAGAATTGAT CATAATGCCG	2400
AATATTTTCA CTTTGAAGGA TGGTATGATG ATCGTCCTCG TTCAATTATG GTGTATGCAC	2460
CTTGTAGAAC AGCAGTGGTC TATGCACTAG TAGACAAAGA AGAAGAAGAA GAAGAAGAAG	2520
AAGAAGAAGT AGCAGTAGTA GAAGAAGTAG TAGTAGAAGA AGAATGAACG AACTTGTTG	2578

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

AATTTYATGG GNAAYGARTT YGG

## CLAIMS

1. Starch extracted from a potato plant and having an amylose content of at least 35%, as judged by the iodometric assay method of Morrison & Laignelet (1983 J. Cereal Science 1, 9-20).
2. Starch according to claim 1, having an amylose content of at least 37%, as judged by the method defined in claim 1.
3. Starch according to claim 1, having an amylose content of at least 40%, as judged by the method defined in claim 1.
4. Starch according to claim 1, having an amylose content of at least 50%, as judged by the method defined in claim 1.
5. Starch according to claim 1, having an amylose content of at least 66%, as judged by the method defined in claim 1.
6. Starch according to any one of claims 1-5, having an amylose content of 35 - 66%, as judged by the method defined in claim 1.
7. Starch which as extracted from a potato plant by wet milling at ambient temperature has a viscosity onset temperature in the range 70 - 95°C, as judged by viscoamylograph of a 10% w/w aqueous suspension thereof, performed at atmospheric pressure using the Newport Scientific Rapid Visco Analyser 3C with a heating profile of holding at 50°C for 2 minutes, heating from 50 to 95°C at a rate of 1.5°C per minute, holding at 95°C for 15 minutes, cooling from 95 to 50°C at a rate of 1.5°C per minute, and then holding at 50°C for 15 minutes.
8. Starch which as extracted from a potato plant by wet milling at ambient temperature has peak viscosity in the range 500 - 12 stirring number units (SNU's), as judged by viscoamylograph conducted according to the protocol defined in claim 7.



9. Starch which as extracted from a potato plant by wet milling at ambient temperature has a pasting viscosity in the range 214 - 434 SNU's, as judged by viscoamylograph conducted according to the protocol defined in claim 7.
10. Starch which as extracted from a potato plant by wet milling at ambient temperature has a set-back viscosity in the range 450 - 618 SNU's, as judged by viscoamylograph conducted according to the protocol defined in claim 7.
11. Starch which as extracted from a potato plant by wet milling at ambient temperature has a set-back viscosity in the range 14 - 192 SNU's, as judged by viscoamylograph conducted according to the protocol defined in claim 7.
12. Starch which as extracted from a potato plant by wet milling at ambient temperature has a peak viscosity in the range 200 - 500 SNU's and a set-back viscosity in the range 275-618 SNU's as judged by viscoamylograph according to the protocol defined in claim 7.
13. Starch which as extracted from a potato plant by wet milling at ambient temperature has a viscosity which does not decrease between the start of the heating phase (step 2) and the start of the final holding phase (step 5) and has a set-back viscosity of 303 SNU's or less as judged by viscoamylograph according to the protocol defined in claim 7.
14. Starch which as extracted from a potato plant by wet milling at ambient temperature displays no significant increase in viscosity as judged by viscoamylograph conducted according to the protocol defined in claim 7.
15. Starch which as extracted from a potato plant by wet milling at ambient temperature, is in accordance with claim 7 and in accordance with any one of claims 8 to 14.
16. Starch according to any one of claims 7 to 15. having an amylose content in the range 35 - 66%, as judged by the method of Morrison & Laignelet defined in claim 1.

17. Starch which as extracted from a potato plant, has a phosphorus content in excess of 200mg/100grams dry weight starch.
18. Starch according to claim 17, having a phosphorus content in the range 200 - 240mg/100grams dry weight starch.
19. Starch according to claim 17 or 18, further in accordance with any one of claims 1 to 16.
20. Starch prepared by physical, chemical and/or enzymatic treatment of a starch initially having properties in accordance with any one of claims 1-19.
21. Starch according to claim 20, being resistant starch prepared by physical, chemical and/or enzymatic treatment of a starch initially having properties in accordance with any one of claims 1-19.
22. Starch according to claim 21, comprising in excess of 5% total dietary fibre, as determined according to the method of Prosky *et al.*, (1985 J. Assoc. Off. Anal. Chem. 68, 677).
23. Use of starch according to any one of claims 1-22 in the preparation or processing of a foodstuff.
24. Use of starch according to claim 23, wherein the starch is used to provide a film, barrier, coating or as a gelling agent.
25. Use of starch according to claim 23, to prepare resistant starch compositions.
26. Use of starch according to any one of claims 1-22 in the preparation or processing of corrugating adhesives, biodegradable products, packaging, glass fibers and textiles.
27. A nucleotide sequence encoding an effective portion of a class A starch branching

enzyme (SBE) obtainable from potato plants.

28. A nucleotide sequence according to claim 27, encoding a polypeptide comprising substantially the amino acid sequence of residues 49 to 882 of the sequence shown in Figure 5.

29. A nucleotide sequence according to claim 27 or 28, comprising substantially the sequence of nucleotides 289 to 2790 of the sequence shown in Figure 5, or a functional equivalent thereof.

30. A nucleotide sequence according to claim 29, further comprising the sequence of nucleotides 145 to 288 of the sequence shown in Figure 5, or a functional equivalent thereof.

31. A nucleotide sequence according to claim 27, comprising the sequence of nucleotides 228 to 2855 of the sequence labelled psbe2con.seq in Figure 8, or a functional equivalent thereof.

32. A nucleotide sequence according to claim 27, comprising the sequence of nucleotides 57 to 2564 of the sequence labelled as psbe2con.seq in Figure 12, or a functional equivalent thereof.

33. A nucleotide sequence according to any one of claims 27 to 32, comprising an in-frame ATG start codon, and optionally including a 5' and/or a 3' untranslated region.

34. A nucleotide sequence according to claim 27, comprising the sequence of nucleotides 45 to 3200 of the sequence labelled as psbe2con.seq in Figure 8, or a functional equivalent thereof.

35. A nucleic acid construct comprising a sequence in accordance with any one of claims 27 to 34.

36. An expression vector comprising a nucleic acid construct according to claim 35.
37. A host cell into which has been introduced a sequence in accordance with any one of claims 27 to 36.
38. An effective portion of a class A SBE polypeptide obtainable from potato plants and encoded by a nucleotide sequence in accordance with any one of claims 27 to 36.
39. A polypeptide according to claim 38, comprising substantially the sequence of amino acids 49 to 882 of the sequence shown in Figure 5, or a functional equivalent thereof.
40. A polypeptide according to claim 38 or 39, comprising the sequence of amino acids 1 to 48 of the sequence shown in Figure 5.
41. A polypeptide in accordance with any one of claims 38, 39 or 40 in substantial isolation from other plant-derived constituents.
42. A method of altering the characteristics of a plant, comprising introducing into the plant a portion of a nucleotide sequence in accordance with any one of claims 27 to 36, operably linked to a suitable promoter active in the plant, so as to affect the expression of a gene present in the plant.
43. A method according to claim 42, wherein the nucleotide sequence is operably linked in the anti-sense orientation to a suitable promoter active in the plant.
44. A method according to claim 42, wherein the introduced sequence comprises one or more of the following operably linked in the sense orientation to a promoter active in the plant, so as to cause sense suppression of an enzyme naturally expressed in the plant: a 5' untranslated region, a 3' untranslated region, or a coding region of the potato SBE class A starch branching enzyme.
45. A method according to any one of claims 42, 43 or 44, further comprising

introducing into the plant one or more further sequences.

46. A method according to claim 45, wherein one or more of the further sequences are operably linked in the anti-sense orientation to a suitable promoter active in the plant.

47. A method according to claim 45 or 46, wherein the further sequence comprises a portion of a class B SBE nucleotide sequence.

48. A method according to any one of claims 42 to 47, effective in altering the starch composition of a plant.

49. A plant or plant cell having characteristics altered by the method of any one of claims 42 to 48, or the progeny of such a plant, or part of such a plant.

50. A plant according to claim 49, selected from one of the following: potato, pea, tomato, maize, wheat, rice, barley, sweet potato, and cassava.

51. A tuber or other storage organ from a plant according to claim 49 or 50.

52. Use of a tuber or other storage organ according to claim 51, in the preparation and/or processing of a foodstuff.

53. A plant according to claim 49 or 50, containing starch which, as extracted from the plant by wet milling at ambient temperature, has an elevated viscosity onset temperature as judged by viscoamylograph conducted according to the protocol defined in claim 7, compared to starch extracted from a similar, but unaltered, plant.

54. A plant according to claim 53, wherein the viscosity onset temperature is elevated by an amount in the range of 10 to 25°C.

55. A plant according to claim 49 or 50, containing starch which, as extracted from the plant by wet milling at ambient temperature, has a decreased peak viscosity as judged by

viscoamylograph conducted according to the protocol defined in claim 7, compared to starch extracted from a similar, but unaltered, plant.

56. A plant according to claim 55, wherein the peak viscosity is decreased by an amount in the range of 240 to 700 SNU.

57. A plant according to claim 49 or 50, containing starch which, as extracted from the plant by wet milling at ambient temperature, has an increased pasting viscosity as judged by viscoamylograph conducted according to the protocol defined in claim 7, compared to starch extracted from a similar, but unaltered, plant.

58. A plant according to claim 57, wherein the pasting viscosity is increased by an amount in the range of 37 to 260 SNU.

59. A plant according to claim 49 or 50, containing starch which, as extracted from the plant by wet milling at ambient temperature, has an increased set-back viscosity as judged by viscoamylograph conducted according to the protocol defined in claim 7, compared to starch extracted from a similar, but unaltered, plant.

60. A plant according to claim 59, wherein the set-back viscosity is increased by an amount in the range of 224 to 313 SNU.

61. A plant according to claim 49 or 50, containing starch which, as extracted from the plant by wet milling at ambient temperature, has a decreased set-back viscosity as judged by viscoamylograph conducted according to the protocol defined in claim 7, compared to starch extracted from a similar, but unaltered, plant.

62. A plant according to claim 49 or 50, containing starch which, as extracted from the plant by wet milling at ambient temperature, has an elevated apparent amylose content as judged by iodometric assay according to the method of Morrison & Laignelet, compared to starch extracted from a similar, but unaltered, plant.

63. A plant according to claim 49 or 50, containing starch which, as extracted from the plant, has a phosphorus content in excess of 200mg/100grams dry weight starch.
64. Starch obtainable from a plant according to any one of claims 49, 50 or 53 - 63.
65. Starch according to claim 64 and further in accordance with any one of claims 1 - 22.
66. A method of modifying starch *in vitro*, comprising treating starch under suitable conditions with an effective amount of a polypeptide in accordance with any one of claims 38 to 41.
67. A potato plant or part thereof which, in its wild type possesses an effective SBE A gene, but which plant has been altered such that there is no effective expression of an SBE A polypeptide within the cells of at least part of the plant.
68. A potato plant according to claim 67, wherein the alteration is effected by a method according to any one of claims 42-48.

## INTERNATIONAL SEARCH REPORT

 Inter. Patent Application No.  
 PC:/GB 96/01075

 A. CLASSIFICATION OF SUBJECT MATTER  
 IPC 6 C12N15/82 C12N15/54 C08B30/04 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

 Minimum documentation searched (classification system followed by classification symbols)  
 IPC 6 C12N C08B A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THE PLANT JOURNAL, vol. 7, no. 1, January 1995, pages 3-15, XP002014042 BURTON, R.A., ET AL.: "Starch branching enzymes belonging to distinct enzyme families are differentially expressed during pea embryo development" see the whole document ---	28-32, 34,39-41
X	DATABASE WPI Section Ch, Week 9442 Derwent Publications Ltd., London, GB; Class C06, AN 94-337418 XP002014047 & JP,A,06 261 767 (MITSUI GYOSAI SHOKUBUTSU BIO KENKYUSHO) , 20 September 1994 see abstract ---	28-37, 39-42, 48-50
-/-		

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \* "A" document defining the general state of the art which is not considered to be of particular relevance
- \* "E" earlier document but published on or after the international filing date
- \* "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \* "O" document referring to an oral disclosure, use, exhibition or other means
- \* "P" document published prior to the international filing date but later than the priority date claimed

\* "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\* "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\* "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\* "&" document member of the same patent family

Date of the actual completion of the international search

23 September 1996

Date of mailing of the international search report

23. 10. 96

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 European Patent Office, P.B. 5818 Patentlaan 2  
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Authorized officer

Maddox, A



## INTERNATIONAL SEARCH REPORT

International Application No  
PC/GB 96/01075

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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